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Crazy wasps: when parasites manipulate the *Polistes* phenotype

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The infection of *Polistes dominulus* wasps by the strepsipteran *Xenos vesparum* provides a suitable case study for exploring parasitic manipulation. One aim of this review is to summarize the life cycle of *X. vesparum*: from infection of immature wasps to the "stylopization" of adults, and from its mating at summer aberrant aggregations of infected wasps to the overwintering of fertilized *Xenos* inside the abdomen of hibernating wasps. The second aim of this review it to highlight how this parasite manipulates the flexible phenotype of the wasp to maximize its own reproductive success.

Parasite-induced behavioural changes: an open question

For over 30 years, the manipulation of host behaviour by parasites has fascinated biologists and parasitologists (*see* Moore 2002 for reviews). From an evolutionary perspective, host's behavioural changes are traditionally seen as a perfect example of the parasite's 'extended phenotype' (*sensu* Dawkins 1982). The debate on this topic "is far from being weak" (Thomas *et al.* 2005), although studies focused on the "adaptive manipulation hypothesis" have decreased since the mid-1980s (Poulin 2000). Recently, this paradigm has been critically reassessed (Moore & Gotelli 1990, Poulin 1995). Indeed, parasite-induced changes in behaviour could simply be (1) the side-effects of infection, (2) the ancestral characters of the parasite, (3) or host adaptations to reduce pathological consequences, with no (or only accidental) adaptive value for the parasite. Altered host behaviour is considered *adaptive manipulation* by the parasite (Poulin 1995) if the manipulation is "complex" and involves a "purposive design", if it independently evolved in many lineages, and if it increases the fitness of the parasite via improved transmission probability, or the fitness of the host via inclusive fitness: the host's sacrifice could help its kin to complete their life cycle.

Many parasites affect their host's behaviour. Most of them — mainly *parasitoid* insects (*sensu* Godfray 1994) — induce *simple alterations*, such as foraging or moving more or less. Although increases or decreases in host activity may enhance parasite transmission, these simple changes may be coincidently beneficial for the parasite, mere by-products of infection. But *complex aberrant behaviours* are less likely to evolve by chance. A notable recent example (Eberhard 2000) is the unusual 'cocoon web' that an orb-weaving spider, when parasitized by an ichneumonid, builds on the night that the wasp larva moults and eats the spider. This novel web provides support for the wasp's pupal cocoon during the heavy rains.

Nevertheless, behavioural changes are often inconspicuous, and the parasite–host interaction very subtle. For example, when the parasite's manipulation *exploits* host's behavioural patterns, the net effect of parasitism may be prolonged host survival or other apparent benefits. As recently pointed out by Thomas *et al.* (2005), "*we have until now only studied the visible part of the iceberg*", i.e. the most obvious altered behaviours of the host. Indeed, the range of subtle modifications may favour parasites, hosts, or both.

The infection of *Polistes dominulus* by the strepsipteran *Xenos vesparum* may provide a case study from which to explore parasitic manipulation. This approach is greatly facilitated by the amount of research previously conducted on *P. dominulus* and on the growing body of research on these widespread macroparasites (in addition to social and solitary Hymenoptera, Strepsiptera infect six insect orders, *see* Kathirithamby [1989]). The aim of this review is to summarize the bizarre lifestyle of this endoparasite and to specifically focus on the behaviour of infected *Polistes* wasps. In my view, the heavily explored topics of relatedness, kin selection, and reproduction at the individual and colony level have marginalized such issues as the natural enemies of *Polistes* nests. This is especially true if parasite-induced behavioural modification occurs away from the nest — the traditional research barycentre — such as at lekking or hibernation sites (but *see* Beani 1996, Dapporto *et al.* 2004).

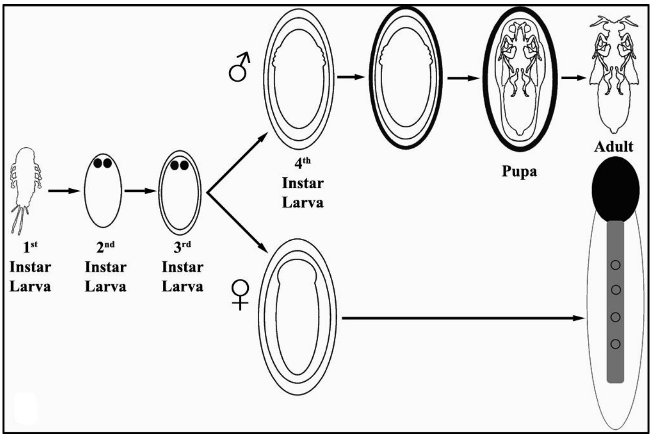

Fig. 1. Scheme of the post-embryonic development of male and female *X. vesparum*. The 1st, 2nd and 3rd instar larvae are found in all larval stages of the host, whereas the 4th instar larva is found in its host's late pupal stage. Endoparasitic development is completed during the host's imaginal stage (1–2 weeks after its eclosion), when *Xenos* terminal instars of both sexes extrude through the intersegmental membranes of the host's abdomen. Note that, after the moult of the 1st instar larva, apolysis is not followed by ecdysis, thus successive instars retain the exuvia of the previous stages. (From Beani *et al.* 2005).

A mysterious cryptic parasite developing inside a well-known host

This enigmatic group of "twisted-winged" parasites — called Strepsiptera — "challenges the biological rule book" (Proffitt 2005), due to several striking features. Most striking, perhaps, is that this parasite has the most extreme sex-dimorphism found in insects, with winged males and larviform endoparasite females who infect different hosts in Mirmecolacidae (Kathirithamby & Hamilton 1992, Kathirithamby 2005). This group also shows "apolisis without ecdysis" (Kathirithamby 1984), meaning that during its endoparasitic development it produces a multi-stratified male puparium or female cuticle, respectively (Fig. 1). In addition, permanently endoparasite viviparous females develop "novel structures" (Kathirithamby 2000). Recent studies described a peculiar "masquerading" strategy for avoiding host immune response (Kathirithamby *et al.* 2003), a bizarre genome, to date the smallest one among insects (Johnston *et al* 2004), peculiar arrangements in mtDNA (Carapelli *et al.* 2006), unusual extra-genital mating modalities (Beani *et al* 2005), and the list goes on. Moreover, the phylogenetic relationships of this order is still controversial (the so-called "Strepsiptera problem", Whiting *et al.* 1997), regardless of the use of molecular and morphological data (Whiting 1998, Huelsenbeck 2001, for reviews), the comparative analysis at the level of sperm ultrastructure (Carcupino *et al.* 1995, Dallai *et al.* 2003) and larvae (Pohl 2002), or the use of fossil strepsipterans in amber (Pohl *et al.* 2005, Grimaldi *et al.* 2005).

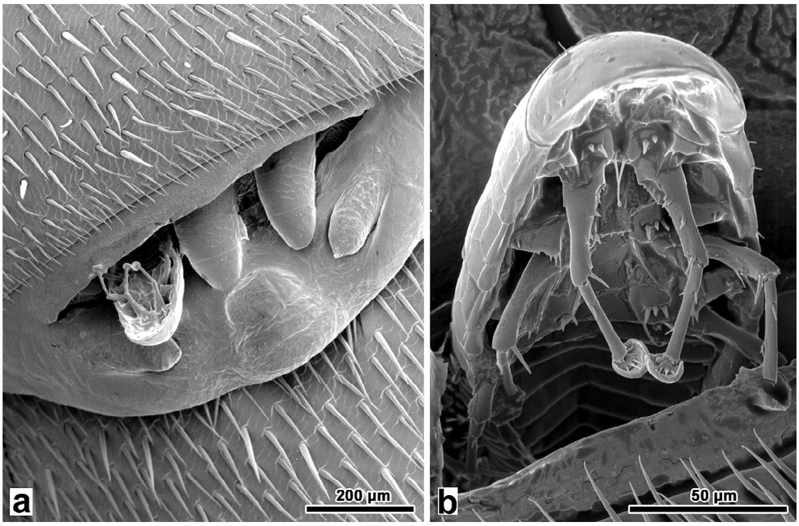

Fig. 2. SEM. — **a**: Three triungulins, the 1st instar larvae, are emerging from the brood opening, in the cephalothorax of a *X. vesparum* female. — **b**: At higher magnification, it is possible to observe the tarsal pad of the anterior legs. (From Beani *et al.* 2005).

First chapter: infection of wasp immatures

Although *Xenos vesparum* was the first strepsipteran described (by Rossi [1793], in *Polistes dominulus* Christ), it is not surprising that studies on the behaviour of parasitized wasps have been so rare. One of the main characteristics of this parasite is its *cryptic nature*, which renders its study difficult. Infection begins with the entry of tiny "*triungulins*" (Fig. 2) — host-seeking 1st-instar larvae (length: 0.50 mm) — into immature stages of the wasp via unmelanized entry holes in the larval cuticle. Then the triungulin moults into an apodous 2nd instar, which is transparent except for its eyes. The successive endoparasitic stages follow without remarkable growth until the late pupal stages of the host, when the wasp is adult-like and the parasite is approximately 4–5 mm (Fig. 1). This "slow growth" could explain the apparent lack of cost of parasitism for immature wasps, in terms of mortality and lost mass (Hughes & Kathirithamby 2005).

Thus, a profitable way to detect parasitism of *X. vesparum* in immatures is by dissecting late instar larvae and pupae. The degree of parasitism may be surprising. In Tuscany, 58% of *P. dominulu*s nests were infected (Hughes *et al.* 2003). The *mean parasite prevalence* — i.e. the proportion of infected larvae and pupae — was 0.16, with a highly variable number of parasites per brood, ranging from 1 to 9 (the *parasite load*). But none of the adults from these heavily infected nests were parasitized at the time of collection (June, i.e. worker emergence phase). A similar trend has been observed across other *Polistes* species from Italy and Mexico. Because infected adults are rarely found on the next, this component of wasp biology remains unappreciated, and will remain so without systematic nest collection and laborious brood dissections.

Unfortunately, this approach overstates effective occurrence of parasitized wasps in the field. In fact, the parasite load in immatures is higher than in adults, which are usually singly infected (Hughes *et al.* 2004a, 2004b). The entry of many triungulins is likely to induce the death of the larval host, although a few super-infected wasps have been collected both in our samples and in *P. annularis* parasitized by *Xenos pallidus* (Dunkle 1979). In the laboratory, the removal by adults of super-infected (with 5 parasites or more) larvae — either alive or dead — has been recorded, as well as successful eclosion of super-infected pupae (L. Beani pers. obs.). In addition, a "local competition for resources" among endoparasites— mainly during the host's pupal phase — may lead to asynchronous development of the endoparasites inside the abdomen. The occasional extraction of both immature and mature parasites from a single wasp (R. Dallai unpubl. data), supports this hypothesis. The label "singly infected" wasp refers only to the parasite that has completed its development, and not all cohabiting parasites.

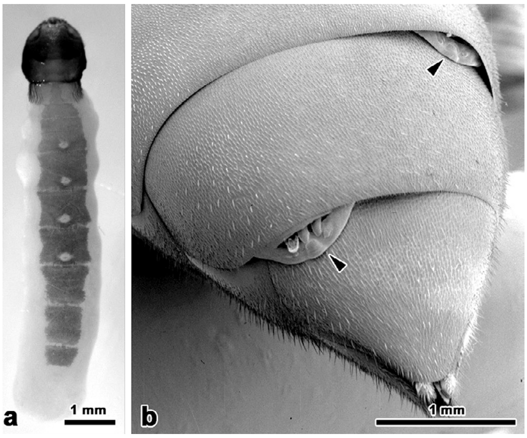

Fig. 3. SEM. — **a**: The neotenic larviform *X. vesparum* female, with the cephalothorax, the dark ribbon forming the ventral canal and the four openings of its ducts. — **b**: Only the sclerotized cephalothorax of two *Xenos* females (arrowheads) extrude, respectively, from the 4th and the 5th tergites of a *P. dominulus* female. (From Beani *et al.* 2005).

Interestingly, *P. gallicus* immatures, from the same area and in the same period (Hughes *et al.* 2003), were more heavily infected than were *P. dominulus* ones (mean parasite prevalence: 0.33; parasite load: 1–15). However, in extensive field collections across seasons, we have never found parasitized *P. gallicus* adults (Hughes *et al.* 2004a). In addition to field evidence, in the laboratory artificial infections of *P. gallicus* nests were conducted by putting 1–3 triungulins directly on larvae (this method was first used by Strambi & Strambi 1973). Endoparasitic instars normally developed until the pupal stage, but no infected adult wasps emerged (R. Dallai unpubl. data). Moreover, artificial infection of *P. dominulus* immatures by means of 1–3 triungulins were usually successful. Thus, *P. gallicus* might represent an unsuitable host for *X. vesparum*.

Second chapter: stylopization of the host, mating and overwintering of the parasite

The terminal instar of *X. vesparum*, when female, does not pupate but becomes a neotenic larviform endoparasite (final size: 7 mm). This is essentially a “bag” of free oocytes and adipocytes covered by multiple cuticles, which protrudes a sclerotized cephalothorax through the intersegmental membranes of a wasp. An extragenital canal opens in this region, where both mating and larval escape occur (Beani *et al.* 2005, Kathirithamby 2005). An inspection of an infected wasp’s abdomen will reveal the presence of a flattened, brown cephalothorax, usually between the last tergites, which partially or totally cover the parasite (Fig. 3). In contrast, the final instar of the male strepsipteran pupates. The adult male (Fig. 4) is a small fly-like insect (approximate length: 3 mm), that is just able to break the cap of his puparium and fly off to search for a receptive mate.

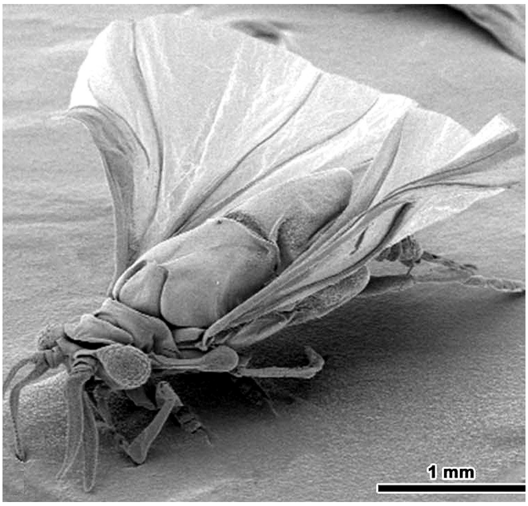

Fig. 4. SEM. A *X. vesparum* male with branched antennae, mesothoracic wings reduced to halteres, and metathoracic twisted-wings. (From Beani *et al.* 2005).

The so-called “stylopization” of the host (Stylopidae is the family of *Xenos*) refers to the characteristic protrusion of the male melanized cephalotheca — or the less conspicuous female cephalothorax — through the wasp cuticle (Fig. 5). But this protrusion, while useful to detect parasitism, results in male-biased estimates and occurs only 1–2 weeks after the emergence of the adult wasp from its cell. This short window of identification is exasperated by the fact that artificially infected wasps leave the colony and remain in the foraging areas of their cages at about 5 days post-eclosion (Hughes *et al.* 2004b), i.e. before any clear signs of stylopization are present. The label *crypto-parasitized* refers to this brief stage of pathology, just before the extrusion of the parasite.

Fig. 5. The 5 black closed puparia of *X. vesparum* males, extruding from the tergites and the sternites of this super-infected *P. dominulus* female, are more conspicuous than the flattened cephalothorax of the female (3rd tergite, arrowhead).

Parasite mating peak occurs between July and mid-August (Hughes *et al.* 2004b) at extranidal aberrant aggregations of infected wasps, which act as vehicles of both puparia and receptive females (*see* Table 1 and the last section). Here, outbreeding and mate location is enhanced, and mate choice may be mediated by sex pheromones produced by Nassonow's glands in mature females (Dallai *et al.* 2004). In these aggregations of infected wasps, aggression is not directed toward fragile males, whereas these "little persecutors" are attacked near nests (*see* Hubbard [1982] for anecdotal first report). Mating is often disturbed by the brushing of the wasp hind legs, thus reinforcing a quick extragenital insemination (Beani *et al.* 2005). Males die 5–6 hrs after emergence, whereas wasps of both sexes, carrying empty puparia, usually die after a few weeks.

Fertilized *Xenos* females, in a singly infected host, *overwinter inside their hosts* (up to 25% of hibernating *P. dominulus* females were infected, Hughes *et al.* [2004a], *see* Turillazzi [1980] for previous data). In the laboratory, 3–4 weeks after winter diapause (Quercioli *et al.* 2000), these permanently endoparasitic females are able to release triungulins and infect new colonies. Phoretic infection of wasp immatures or, more likely, direct release of triungulins on nests (*see* Hughes *et al.* 2003), may occur until July, when all overwintered parasitized wasps die, at least in the lab. Deviations from this simple schedule may include, for example, a bivoltine cycle of the parasite (i.e. two mating peaks, involving first workers, then sexuals as vectors of the parasite), but it has not been documented in our populations.

In conclusion, seeking stylopized wasps at summer extra-nidal aggregations is "the best of a bad job". Low levels of parasitism were found among adults collected at foraging sites (1 out of 63) and at water sources (4 out of 88) between the spring and summer, as well as on nests (Hughes *et al.* 2004a). Collection of *P. dominulus* nests in the same area as our previous nest collection and dissection, but later in

Table 1. Behavioural changes in *P. dominulus* wasps affected by *Xenos vesparum*: first data.

Aberrant behaviours	Host sex and caste	Parasite	References
Summer extranidal aggregations on lek and pre-hibernant sites	*P.dominulus* females, workers and sexuals (2% males), inactive	*Xenos* males and females, one per host (81%), extragenital insemination	Hughes *et al.* 2004a, 2004b, Beani *et al.* 2005
Early colony desertion, no helping at the nest, no eviction by healthy wasps	*P. dominulus* workers artificially infected in lab (large cages), crypto-parasitized	*Xenos* males and females	Hughes *et al.* 2004b
Prolonged hibernant groups (until April)	*P. dominulus* females, abundant fat bodies development	1–2 fertilized *Xenos* females per host, embryos	Beani *et al.* 2004a
No stable association with founding wasps, no nesting nor helper activity	*P. dominulus* overwintered females	1–2 fertilized *Xenos* females releasing triungulins	Pardi 1942, Beani *et al.* 2004b, Quercioli *et al.* 2000

the season (July–beginning of August, i.e. sexual emergence phase), revealed only 61 infected wasps out of 894 dissected adults, and 53 were crypto-parasitized (Hughes *et al*. 2004b). Detection of infected colonies — without dissection of both immatures and adults — is accidental and unreliable due to the very limited temporal window in which parasitized wasps live in their colony (about 1 week), and their status of crypto-parasitized animals. Due to the traditional focus on individual wasps interacting on the nest, the pressure exerted by *Xenos* parasites on *Polistes* life history has been undervalued!

When the *Polistes* fortress is threatened

Enemies and defence of the colony

Social insect colonies are ideal for parasites because of the high density of similarly genotyped hosts in a nest. However, invasion of the colony is difficult. Schimd-Hempel (1998) employs the successful metaphor of the "factory fortress" (*sensu* Wilson 1971) to emphasize this. Firstly, colony life may evolve to resist to parasites. Secondly, abundant and persistent colonies of social insects — more or less genetically related — represent a prime target for a lot of parasites. A useful synopsis of studies is provided in Appendix 2 (Schmid-Hempel 1998), where *Polistes* nests and wasps are listed as a target of fungal diseases, of mites (mainly phoretic or saprophagous, living on debris or nest provisions: Eickwort [1990], sometimes truly parasitic: Nelson [1968]), and of a wide variety of insects, including Hymenoptera (mostly Chalcididae, Ichneumonidae, Mutilidae), Diptera (Sarcophagidae, Tachinidae), and Lepidoptera.

The brood of social wasps that construct unenveloped combs — like *Polistes* — are attacked by parasitoid insects, which oviposit on the cell walls, eventually consume the pupal host, and then pupate themselves (reviewed in Nelson 1968, Makino 1985, Yamane 1996, Hughes *et al*. 2003). Some families of "predaceous moths" (Jeanne 1979) consume host tissue and meconia by tunneling from cell to cell. Larger colonies of *P. exclamans* were more likely than small colonies to be attacked by parasitic wasps and by a pyralid moth (Strassmann 1981). Thus, small colony size (but not polydomy, *see* Strassmann 1981), multiple comb construction (Jeanne 1979), nest envelops in swarm-founding Polistinae (Jeanne 1991), all seem to provide some defence against parasitoids and predators. These characteristics, of course, decrease production of workers and sexuals for the colony. In addition, the "parasite dance" and attentive search for parasitoids over the nest, if parasites are detected near the colony (West-Eberhard 1969), represent perhaps the most peculiar behaviours performed by *Polistes* when their "fortress" is attacked.

Parasitic castration and behavioural changes induced by Strepsiptera

Strepsiptera are promising candidates for the study of host manipulation. These macroparasites are able to exploit the reproductive organs of the host. They are *parasitic castrators* (*sensu* Baudoin 1975), that is, regardless of the parasite load, stylopized *Polistes* develop filiform ovaries if female and do not develop testes if male (preliminary data in Beani *et al*. 2004b). Changes in haemolymph proteins do not compromise wasp longevity (Strambi *et al*. 1982), as would be expected in case of parasitic castration: "*by attacking non-vital organs, castrators do not reduce the host life span, and they can obtain a high transmission rate without trading off longevity*" (Poulin 1998). If resource allocation shifts from reproductive organs to energy reserves, the hosts become efficient vehicles for parasite survival and transmission.

However, in our system, the sex of the parasite directly affects resource allocation, and thus the life span and "caste fate" of the host. Wasps parasitized by *Xenos* females overwinter with healthy gynes, while wasps infected by males disappear between September and October (Hughes *et al*. 2004b). In a sample of 88 hibernating stylopized wasps (Hughes *et al*. 2004a), 60 were singly parasitized, 9 had 2 parasites and one had 3 female *Xenos*; the remaining 8 wasps contained one female and one puparium (empty or with a dead male inside). The paucity of fat bodies, which characterizes wasps containing male *Xenos* in summer collections, is likely to decrease their life span (L. Beani unpubl. data), although storage proteins might also play a role (Hunt *et al*. 2003).

In our population, stylopized wasps clustered in inactive groups until the beginning of April, while healthy gynes scattered to occupy good nesting sites at the beginning of March (2004, $N = 129$, repeated collections under the tiles of two roofs; Beani *et al*. 2004a). This may represent a "waiting strategy" within an aposematic defended herd for both mature larvae and triungulins. When parasitized and unparasitized wasps were caged together, stylopized wasps did not engage in colony work, but instead rested in a corner and were ignored by foundress, auxiliaries, and healthy wasps not involved in nesting activity (Quercioli *et al*. 2000, Beani *et al*. 2004b).

Previous reports generally emphasize the "*sluggish disposition of stylopized individuals*" (reviewed by Salt 1927), but a few studies account for *aberrant behaviour*. For example, the solitary bee *Andrena* undergoes morphological as well as behavioural changes: the pollen-collecting apparatus of the female is reduced and, if infected by a *Stylops* female releasing larvae, her "relatively slow" flight becomes fast. The stylopized bee, moving "rapidly from flower to flower", drags its abdomen through the stamens and spreads triungulins, the tiny pests. In this way, other foraging bees become vectors for infection (triungulins are taken into their crop, *see* Linsley & McSwain [1957]).

When it comes to social hymenopterans, these macroparasites seem be able *to break all caste roles*. In ants, unhealthy workers become diurnal, abandon their nests, and climb high on grasses and bushes (early reports in Oglobin [1939]). These behavioural changes are likely to occur at the emergence of male strepsipteran (Kathirithamby 2005). Unusual short-range "migrations" of overwintered stylopized *P. dominulus* wasps, from one nest to another, were first noted in spring by Pardi (1942). These individuals never worked, but rather rested on the posterior face of different combs and were objects of occasional attack but usually ignored. More recently, Makino and Yamashita (1998) found high rates of workers among hibernating *Vespa* females, but only if parasitized by one *Xenos moutoni* female. These examples attest to the fact that this parasite changes the rules of the game!

Stylopized "crazy" wasps

Table 1 summarizes the aberrant behaviours of *P. dominulus* wasps recently recorded in the field and laboratory. In this host–parasite system, instead of performing novel behaviours, the host performs its usual behavioural patterns, but utterly inappropriate to the season and/or the caste. The most conspicuous phenomenon (*see* Hughes *et al*. 2004b) is the summer *extra-nidal aggregations of inactive stylopized female wasps*. These wasps have deserted the colony at the peak of its development. The mean size of these clusters is 12–14 females and their turnover is high. Parasite prevalence ranges from 0.98 in July to 0.64 in September, when healthy gynes and unhealthy wasps clump together in pre-hibernation groups. These peculiar aggregations, which are present for days at the same site (one leaf was occupied for 36 days!) and on subsequent years (a vineyard was occupied from 1998 to 2006), are *mate-encounter points for the parasite*, not for the castrated host. As a further exploitation of the host habitat choice, these aggregations are *non-randomly located* at traditional lekking and hibernation sites (prominent vegetation, buildings with high nest densities etc).

Last, but not least, these aggregations are *initially — and mostly — formed by workers*, not by sexuals. In absence of clear-cut morphological and behavioural caste cues, this hypothesis is supported by some indirect evidence. Firstly, extra-nidal clusters are formed by artificially infected workers inside large cages (Hughes *et al*. 2004b). Secondly, data was collected on clusters of stylopized *P. dominulus* females located on vegetation during mid-June 2003, which is too early for sexuals. Finally, data show a very low rate of drones (2% or less) in summer aggregations. Due to the entry of triungulins in wasp larvae of both sexes, and the successful emergence of stylopized males in lab (Strambi *et al*. 1982, L. Beani pers. obs.), the most parsimonious explanation for this female-biased sex-ratio is that the main target of triungulins are workers rather than sexuals (at least in our study population and species; but *see* Dunkle [1979] for stylopized sexuals in *P. annularis*).

Parasitic castration does not seem to promote social behaviour in *Polistes* wasps (Hughes *et al.* 2004b, Beani *et al.* 2004b) as hypothesized by O'Donnell (1997), who suggested that such reproductively handicapped females might contribute to the reproductive success of relatives by advantageously acting as workers. O'Donnell (1997: p. 692) also notes, however, that "*in order for parasitic castrators to affect reproductive division of labour, the degree and type of damage of the host must allow for the expression of normal working behaviour*". This condition does not appear to be met in stylopized *P. dominulus*. In *Polistes* normal worker behavior depends on juvenile hormone (JH) production (Giray *et al.* 2005). The stylopization syndrome — aggregation formation and non-worker behavior — resembles the prehibernation state of overwintering wasps, which are in a diapause ovarian state that can be broken by application of JH (Bohm 1972).

The mechanism of the parasites' behavioral manipulation involves JH production: a suggestion confirmed by the work of Strambi and Strambi (1973), who demonstrated decreased size of the JH-secreting organs (corpora allata) in stylopized females. When active corpora allata from locusts were transplanted into females parasitized by *X. vesparum,* ovarian development was stimulated in the parasitized wasps (Strambi & Girardie 1973) suggesting that it is JH production, not sensitivity to the hormone, that is altered by infection. The parasites, then, have performed a *natural experiment* showing that both the queen phenotype (ovarian development) and worker behavior in *Polistes* depend upon persistence of JH production, as suggested by the split- or dual-function hypothesis for JH (West-Eberhard 1996). According to that hypothesis, a completely castrated *Polistes* female, with extremely depressed JH production, should neither reproduce nor work. Both worker and queen phenotypes are derived from the (presumably) hormonally driven reproductive cycle of ancestral solitary females that performed either egg-production or brood-care functions.

From colony perspective, early nest desertion by workers could be interpreted as an "altruistic act" to reduce the infection of kin, although only wasps carrying *Xenos* females — with triungulins — are vectors. This category of wasp was never found in aggregations (neither on the nest nor in the field after mid-July). Nevertheless, lazy stylopized females represent a cost to the colony; thus, their desertion provides some limited benefit for their relatives.

These aggregations roused the curiosity of an extraordinary observer of *Polistes* habits, Bill Hamilton (Hughes 2002). In August 1998, in the surroundings of my house (15 km from Florence, Chianti hills), Hamilton discovered "*aestivation/hibernation gatherings*" of stylopized wasps. He felt that this was "*inappropriate to the time of the year.* [...] *A more inhospitable site for waiting the summer out than the 'oven' which a crack between the tiles of your roof must become under the late Florentine summer sun, is hard for me to imagine!*" He repeatedly encouraged me to study the behaviour of these aggregations of unhealthy wasps, which reminded him a similar phenomenon observed in the tropics in *P. versicolor* (*see* Hughes 2002). "*I think it could be exciting to look for ways the parasites may affect the behaviour of wasps in ways advantageous to them*". In two bright letters about this case-study (e-mails to L. Beani on 3 September 1998 and 21 March 1999), Hamilton mainly stressed both host-parasite coevolution and behavioural manipulation topics. "*After all, if parasites 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 behaviour*" (W. D. Hamilton pers. comm.; *see* also Summers & Leighton John [2001] and, for extensive quotations and comments, Kathirithamby [2005]).

Hamilton often wondered about the puzzling life-cycle of this parasite: "*if triungulins are being transferred in late summer when no larvae are available, is it really possible to imagine these minute delicate animals surviving until nests are well started next spring?*" The overwintering of host and parasite in mixed groups of healthy and unhealthy wasps, which precedes the spring embryo development (R. Dallai unpubl. data), and the release of triungulins from

May to mid-July, answers the last question. This also raises once again the question of *parasitic exploitation of host behaviour*, i.e. the adoption of winter diapause. But many chapters of this story are still obscure. Some of the unexplored topics in the *Xenos–Polistes* system relate to the physiological basis of host behavioural changes (after the excellent pioneer studies by Strambi & Strambi [1973], Strambi *et al*. [1982]), to changes at the level of epicuticular hydrocarbons, venom volatiles of stylopized wasps and further chemical cues (*see* Beani *et al*. 2005, Bruschini *et al* 2005, Dapporto *et al*. 2006), to the intimate relationship between the parasite and the host's immune system, to analysis of the "horizontal transmission" of parasites from one social insect colony to another (*see* Hughes 2005), and finally to the impact of strepsipterans in North America populations of *P. dominulus*, a recent invasive species (Pickett & Wenzel 2000, Liebert *et al*. 2006).

Finally, we have to find a new label for these "crazy wasps", who are neither workers nor gynes, who never help at the nest, but who cluster at traditional lek- and pre-hibernation sites, and overwinter if infected by only one *Xenos* female. This parasite offers the opportunity for a *natural manipulation experiment* of caste determination in a primitively eusocial paper wasp, where parasites irreversibly (Strambi *et al*. 1982) inhibit the host's gonadal development and act as "modifiers" of a very plastic phenotype (*sensu* West-Eberhard 2003).

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