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Cost of strepsipteran macroparasitism for immature wasps: does sociality modulate virulence?

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An important factor modulating parasite virulence is the level of extrinsic mortality experienced by hosts. Where it is high, parasites are expected to grow or reproduce quickly to complete their lifecycle before their host is killed, whereas virulence is expected to be less under low extrinsic mortality, where growth/reproduction can be slower. A prominent example of a low mortality environment for parasites are immature social insects. Here we examined the cost of parasitism, i.e. virulence, experienced by larval and pupal stages of *Polistes* wasps following infection by endoparasitic Strepsiptera (under starvation conditions). We found that there was no difference in virulence between infected and uninfected individuals for the seven days following infection; either measured as host mortality or mass loss. Likewise, there was no observed cost of parasitism during the first seven days of the pupal stage of the host. Growth of the endoparasitic stages appeared the same between starved laboratory individuals and field caught samples. Strepsipteran parasites apparently enter a lag phase until the later stages of host pupal development, which we speculate reduces the negative impact of parasitism during the hosts' critical developmental stages. Our results highlight the need for further inquiry into the influence of sociality upon the evolution of parasite virulence.

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Virulence is the reduction in host fitness which accompanies parasitism (Bull 1994, Read 1994, Ewald 1995) and is not strictly adaptive for either parasite or host, but rather is a result of the interaction between the two parties (Ebert and Hamilton 1996). The assertion that virulence increases with extrinsic host mortality rate is an intuitively powerful idea which has received wide theoretical consensus (May and Anderson 1983, Sasaki and Iwasa 1991, Lenski and May 1994, Ebert and Weisser 1997, but see also Williams and Day 2001). Briefly, parasites exploiting hosts which occupy high extrinsic mortality habitats should develop quickly in order to complete their life-cycle before host death. Therefore, rapid parasite development should result in increased cost, or virulence, for the host. One troubling aspect of the role of extrinsic mortality upon virulence is the absence of empirical support; which is sometimes reflective of difficulties in addressing the role of mortality (Ebert and Mangin 1997). Supportive evidence that high extrinsic mortality results in rapid development and increased virulence does come from observed virulence among endoparasitoids of herbivorous insects. Where hosts occupied a low extrinsic mortality habitat (plant galls or roots) the parasitoids developed more slowly, whereas parasitoids of exposed, foliar feeding hosts developed rapidly (Harvey and Strand 2002).

Whilst concealed hosts in galls and/or roots are a relatively safe environment for parasites, one other group, the social insects, present a special case in considering parasite virulence. Here, a large proportion

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of individuals, i.e. immature forms, do not leave the confines of the colony and are protected from predation by adaptive nest architecture as well as altruistic kin capable of self-sacrifice in the face of threats (Wilson 1971). Therefore, with the exception of catastrophic events, an immature social insect is assumed (Wilson 1971, Jeanne 1975) to suffer negligible extrinsic mortality in comparison to immature non-social forms and represents a very safe environment for the development of parasites. In view of the fact that the majority of animals are insects (May 1990) and the majority of those, in terms of biomass, are social (May 1989, Hölldobler and Wilson 1990, Jeanne 1991, Tobin 1991), then it is apparent that the social insect colony deserves further attention in considering the evolution and expression of parasite virulence. Particularly, there is a need to identify parasite taxa that may serve as models for the empirical assessment of ecological factors impinging on the evolution and expression of virulence (sensu Harvey and Strand 2002)

The macroparasite insect group, Strepsiptera, is a promising candidate to empirically examine virulence because of its recently identified tractability to laboratory manipulation (Hughes 2003) and very broad host range (34 families in 7 orders are parasitized, Kathirithamby 1989). The Strepsiptera are highly virulent parasites for solitary and social Hymenoptera because they castrate their adult hosts (Salt 1927, Brandenburg 1956, Strambi and Strambi 1973, Maeta and Kurihara 1999). Further, in social forms their presence inhibits normal social behaviour and ultimately leads to nest desertion (Hughes et al. 2004). What is less understood is the cost of parasitism for immature social insects following infection by Strepsiptera. Infection begins with the entry of the free-living, 1st instar larva into a larval host, and less frequently into a host egg. There follows successive endoparasitic stages until the host attains adulthood. The terminal instar of the parasite, if male, forms a puparia, after it extrudes its anterior region through the intersegmental membranes of the abdomen. The terminal instar of the female strepsipteran does not pupate (Kathirithamby 2000) but becomes a permanently parasitic neotenic adult female (after it too has extruded its anterior region through the host's cuticle). The male strepsipteran emerges from its puparia for its short adult life (usually <5 h) in which time it finds and inseminates a female through an opening in her extruded anterior region (the cephalothorax). A deviation from the standard lifecycle occurs in the family Myrmecolacidae where males parasitize ants whilst the females parasitize orthopterans (Ogloblin 1939, Hughes et al. 2003b, Kathirithamby and Johnston 2003).

Here our aim was to artificially infect immature social wasps *Polistes dominulus* (Christ) with the strepsipteran parasite, *Xenos vesparum* Rossi, in order to examine levels of virulence. We adopt the starvation paradigm (Jokela et al. 1999, Brown et al. 2000), which ensures that the cost of parasitism is not masked due to compensation under ad libitum food conditions. We measure direct mortality as well as assessing mass loss as a surrogate of fitness (Read 1994). Because an important source of virulence is parasite growth we compared the growth schedules of artificially reared endoparasitic stages under zero food conditions with field sampled individuals (which we assumed received nourishment), to examine if they are dissimilar. We also checked hosts for signs of immune defence (encapsulation of 1st instar larvae). In contrast to their effect on adult hosts our results showed that strepsipterans do not impose any cost upon their immature hosts (using our measurement of virulence). We discuss this avirulence with respect to the impact sociality may have upon parasite virulence.

Material and methods

Host rearing

Early stage colonies of *Polistes dominulus* were collected from an area outside Florence, Italy in May 2002 (43°45′N, 11°18′E) and maintained in Oxford under standard conditions (490 cm^3 plastic cages, 26–28°C and 60% RH with a 14L:10D light regime). Colonies were provided, ad libitum, with water, sugar cubes, fly larvae *Musca domestica* L. (as a source of protein for developing brood) and paper for nest construction. To infect wasp larvae they were first removed from the nest using a blunt watchmaker's forceps (following removal of adults by placing the nest in the fridge at 4°C). Larvae were then weighed using a microbalance (SLS, Oxford, UK) with an accuracy of 0.1 mg. Individuals to be infected were chosen randomly by tossing a coin. Following infection (below), both control and experimental larvae were placed in artificial cells (glass tubes, 5 cm long with 1 cm diameter, lined with paper). A plug of cotton wool was placed into each tube to prevent the larva falling out. Each tube was placed horizontally into an eppendorf tray and the tray placed into a seedling growth chamber into which wet sand had been placed (26–28°C with 75–85% RH and 14L:10D light regime). The high humidity minimised larval water loss. Each day (1600–1800 GMT), for seven days post infection, the larvae were removed from the tubes and mass recorded (blind to parasitism state). Mass loss was considered a surrogate of virulence (Read 1994). Removal and weighing was performed with a larval forceps and took less than one minute for each larva. In some cases terminal wasp instar larvae (5th instar) unexpectedly entered the pupal stage despite starvation and the opportunity to record mass loss in the first seven days of the pupal stages was taken. The length of the pupal stage in *P. dominulus* is between 13–16 days (Pardi 1946).

Infection of larvae

Adult female *X. vesparum* (which are permanently parasitic) were collected with their female *P. dominulus* hosts, from overwintering groups in December 2001, from the same area as the nests and maintained under similar conditions. The 1st instar strepsipteran larvae, which are the infective stage, were collected as they emerged live from brood canal of the female's cephalothorax (the wasp was held between thumb and forefinger under a dissecting microscope). The 1st instars were picked up and transferred to the body of a larval wasp using a hair attached to a forceps. An individual was considered successfully infected when the 1st instar fully entered the host (after approximately 2 h). Once this happened the infected larvae was transferred to the artificial cell and maintained without food. Control wasps were sham treated and placed in their glass tube at the same time as infected individuals. We infected each larva with one 1st instar *X. vesparum* because, based upon previous collections, we concluded that the majority of adult hosts on the nest (88.5%, n = 61, unpubl.), or away from the nests (81%, n = 254, Hughes et al. 2004) were singly parasitized. This is considered the most regularly encountered parasite level. Further, single-infections controlled for an effect on virulence due to parasite competition.

Growth of endoparasitic stages

Some of the above larvae, which were given no food, were dissected after 14 days and the endoparasitic strepsipteran larvae stages present were measured using a 1 cm graticule at 10 × magnification on a dissecting microscope. Endoparasitic stages from immatures which pupated during the course of the experiment were also measured and in this case the hosts were sacrificed between 13–17 days after infection. (The increased time, here and above, was allowed to maximise the supposed effect of starvation on the hosts and parasite). Samples of endoparasitic stages from hosts in field-collected nests (collection details in Hughes et al. 2003a) were also measured. The field collected colonies were sacrificed within two hours of collection and the sampled immatures were assumed to have received normal food levels (i.e. not starved). For technical reasons we could not establish a laboratory control (i.e. uninfected but fed) because feeding larval wasps once they were removed form the nest was impossible. No current work exists on larval provisioning rates in field colonies of *Polistes*: however, long periods of starvation during the early phase has not, to our knowledge, been mentioned in the literature. That larvae from field nests were not starved is a qualified assumption until such data becomes available.

All statistics were performed using SPSS version 9.0. Because the initial weight of larvae extracted from the nest was variable we used ANCOVA analysis with mass on day zero as a covariate (Raubenheimer and Simpson 1992). We treated the colony as a fixed effect. The mass on day zero and subsequent days were normally distributed. Means are presented ± SE and tests are two-tailed. Power analysis followed Cohen (1992).

Results

Mortality and weight loss

A total of 106 larvae were successfully infected and 108 were established as controls from 10 colonies. Successful infection occurred when the 1st instar larvae of *X. vesparum* were observed to fully enter the host through the cuticle. Mortality was very low despite the starvation conditions; of the 214 immatures used in this study, 6 infected and 9 uninfected individuals died before the 7 days had elapsed (Yates corrected $\chi^2 = 0.27$, 1 df, $p = 0.60$). The mean time until death was 3.04 ± 0.77 days for uninfected individuals versus 3.17 ± 0.70 days for infected individuals (Mann–Whitney U-test = 23.5, $P = 0.07$, n = 9 controls and n = 6 infected). Thus, daily mass recording occurred for 100 experimental and 99 control immatures.

There was no effect of the colony of origin or parasitism state on weight loss over the seven days of observations (repeated measures ANCOVA, Colony, $p = 0.57$, State, $p = 0.70$, Table 1). In five out of ten colonies the trend was for increased weight loss among unparasitized individuals (unpubl.). Parasitized individuals did not lose more weight than unparasitized ones (Fig. 1, Table 1). Unsurprisingly, an individual's mass at the start of the experiment, the covariate, had a significant effect on subsequent mass recordings (Table 1), as did the host instar stage. There was a highly significant relationship between mass and instar stage prior to infection (linear regression $r^2 = 0.71$, n = 199, $P < 0.0001$). For such

Table 1. Repeated measures ANCOVA table for the effect of colony source (fixed effect), parasitism state and host stage on mass loss for all immatures and individuals which pupated (see text).

	df	F	P
Immature wasps			
colony	9,198	0.85	0.57
parasite state	1,198	0.15	0.70
host stage	2,198	11.63	<0.001
initial mass (covariate)	1,198	449.45	<0.001
Pupal stage wasps			
parasite state	1,54	0.001	0.98
initial mass (covariate)	1,54	307.00	<0.001
days (covariate)	1,54	1.704	0.20

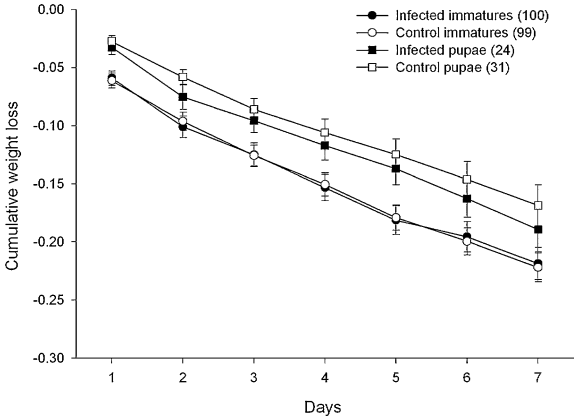

Fig. 1. The cumulative weight loss (mean proportion ± SE) for parasitized and unparasitized immature *P. dominulus* for the seven days following infection and the cumulative weight loss over seven days post-pupa formation according to parasitism state. Values in parentheses are sample sizes.

sample sizes (n = 100 infected and 99 control) the power was very high at moderate, 0.5, and large effect, 0.8, sizes (post-hoc power test 1- = 0.97 and 1.00 respectively) but at low effect size, 0.2, power was relatively low (0.40).

During the course of the experiment some 5th instar wasp larvae unexpectedly became pupae (24 infected and 31 uninfected individuals; Yates corrected $\chi^2 = 0.41$, 1 df, $p = 0.65$) and this allowed the effect of parasitism on weight loss to be assessed for this particular host stage. The mass on day one of pupation (following voiding the gut) was used as a covariate and weight loss recorded for seven days after this date. As larvae entered the pupal state at various times following infection, or sham treatment, the number of days post infection/sham treatment was also used as a covariate. The mean time at which pupation began was 3.29 ± 0.03 days following the start of the experiment. For seven days following the start of pupation there was no difference in weight loss between infected and uninfected individuals (Table 1). The power at the observed sample sizes (n = 24 infected and n = 31 controls) was low for moderate and small effect sizes (0.57 and 0.17 respectively) but high for large effects (0.89).

Growth of parasites within hosts

Once the parasite entered the host it underwent minimal growth while the host remained a larva, and after 14 days post infection in the lab the mean size of *X. vesparum* larvae was 0.58 ± 0.02 mm (n = 7). This was not significantly different from seven randomly selected parasites recovered from host larvae within field collected nests (0.50 ± 0.06, Mann–Whitney U-test, U = 20.5, p = 0.62). The field nests were sacrificed within two hours of capture and are assumed to have consumed food at normal levels (Methods). Where the host became a pupa during the course of the observation, it had reached 'late stage' of pupation (when the pupa is fully formed and has tanned, yellow/black coloured cuticle similar to that in adults). Here, the *X. vesparum* larvae did grow; mean size = 4.30 ± 0.60 mm, (n = 5, mean time since infection was 15.0 days ± 0.71). This was not significantly different from the mean size of endoparasitic stages from field-collected pupae, which can be visually determined to be at the late stages of pupation and similar to those from the lab (3.91 ± 2.08 mm, Mann–Whitney U-test, U = 6.0, P = 0.413).

Discussion

For immature *Polistes* hosts parasitized by Strepsiptera in the seven days following infection it appears that there was no apparent cost of parasitism, as measured through mortality or mass loss (Fig. 1, Table 1). In fact, for half the colonies examined the trend was for increased mass loss by controls (unpubl.). Similarly, when only pupal hosts were examined (first seven days of the pupal stage) there was no cost due to parasitism, in terms of mass loss (Fig. 1, Table 1; we did not record mass loss in the later stages of the host pupal stage). On a priori grounds we would not expect a parasite, which is so dependent upon an adult host for lifecycle completion, to cause the death of the immature host. However, our experiment was conducted under extremely stressful conditions of starvation which has previously been very useful in highlighting the costs of both parasitism (Jokela et al. 1999, Brown et al. 2000), and immune defence (Moret and Schmid-Hempel 2000), so some differences between treatments was expected. Therefore, why are Strepsiptera apparently so avirulent for their larval and early pupal stage host wasps?

One obvious reason is that hosts did not resist infection because if they did, the assumed high cost of immune defence would have been visible under stressful conditions, such as starvation (König and Schmid-Hempel 1995, Ilmonen et al. 2000, Moret and Schmid-Hempel 2000). An ability to mount a humoural defence, i.e. to encapsulate 1st instar larvae, is known to occur, albeit very occasionally, for *P. dominulus* (Hughes et al. 2003a) but it was not observed during daily mass recordings (i.e. haemocytic capsules visible through the cuticle), or during subsequent dissections. The ecological immunology of this host–parasite system is beyond the present scope but it is worth mentioning that some suppression of immune function by the strepsipteran must occur as the entry hole made by the 1st instar does not melanise, but puncturing the cuticle with an inanimate object causes extensive melanisation (D. P. Hughes, pers. obs.). Recently Strepsiptera have been shown to posses a novel mechanism which is

suspected to assist in evading detection by the host; they wrap themselves in host tissue during entry, forming a 'bag' of host tissue around themselves (Kathirithamby et al. 2003).

A probable explanation for the avirulence observed in this study might be the lack of growth (i.e. increase in length) by the parasite following host entry. Comparisons between laboratory-infected and field-caught hosts showed no significant difference in the size of the strepsipteran endoparasitic stages. This was true for both larval and pupal stage hosts. This suggests that laboratory starvation did not alter the normally observed growth schedule of the parasite. Where late pupal stage hosts were dissected, the parasite had achieved a mean size of 4.30 mm after approximately 15 days post infection; but for hosts which remained at the larval stage, the size achieved by their endoparasites was only 0.58 mm after 14 days post infection. (The size of 1st instars is approximately 0.50 mm, including the setae and the final size of adult females, the larger sex, is approximately 7 mm). All pupal hosts were in the final stages of pupation and resembled adult wasps. We speculate that once the 1st instar enters the larval host, and moults to a 2nd instar, growth is halted until the host enters the middle or late stages of pupation. Despite no apparent growth, i.e. increase in body length, we cannot state that internal development of the strepsipteran larvae does not occur: for female strepsipteran larvae the paired ovaries begin to break up in the late 2nd instar stage and development of the oocytes begins in the 3rd instar stage (Kathirithamby et al. 1990). The lack of growth achieved by the strepsipteran might be an adaptation to reduce host-stress during the critical period of early pupation in the host when many major changes occur, as well as possibly reducing parasite-associated deformities to the adult wasp body plan due to the movement of a large endoparasite within the haemocoel. Further, since development must be completed in the host abdomen, a lag phase may be necessary until the larviform host forms a distinct abdomen. The ability to enter a lag phase until a propitious time at which to commence development is an adaptation conditional upon the host's ecology. Such lag phases occur in parasitoids (Salt 1941, Godfray 1994) but importantly no parasitoid, that we know of, infects larval insects and then completes development in the adult insect host–parasitoid lag phases exist to allow the host to acquire more resources necessary for the parasitoid's development (either directly or after diapause). By contrast female strepsipterans, which are not parasitoids (Godfray 1994), require the host to reach adulthood as this host stage is required for movement, feeding, diapause and reproduction (adult male strepsipterans, which do not feed or enter diapause exit the adult host).

Strepsiptera infecting holometabolous insects, i.e. those which have a pupal stage, such as Hymenoptera, are unique in that they infect the immature stage and remain associated with the same individual until it matures to the disparate adult stage: the only parasitic insects which do so! Similarly those infecting hemimetabolous insects (no pupal stage) infect nymphal stages and remain associated into adulthood. Besides hymenopterans, the dipterans are the only other holometabolan order to be infected by strepsipterans. Interestingly, Strepsiptera infecting the oriental fruit flies (Tephritidae: *Bactrocera*) parasitize the adult only after it has emerged from the pupa (i.e. the teneral stage, R. Drew, pers. comm., Drew and Allwood 1985). It is noteworthy that mortality of juvenile fruit flies which live on fruit, is very high; it is reported that up to 70% of larvae die due to fruit eating by birds and rodents (Drew 1987). The lower extrinsic mortality level experienced by immature hymenopteran hosts is due to adaptive architecture and/or protection by siblings, and we suggest that Strepsiptera have exploited this and modulated growth rates and hence virulence, thus enabling them to infect the larval stages and remain endoparasitic till the host reaches the adult stage.

However, our results stand in isolation and as a single system study cannot test the 'slow growth- high mortality hypothesis' (Clancy and Price 1987). Our work is important in that it shows that a macroparasite which is highly virulent for adult wasps (Strambi and Strambi 1973, Hughes et al. 2004) is avirulent for its larval stage host. Because strepsipterans are the only parasitic insects (including parasitoids) to sequentially parasitize disparate stages of the same holometabolan host, they offer a unique opportunity to test the effect of host life history on virulence. Further, their broad host range (solitary to social hymenopterans and solitary members of six other orders), combined with their tractability to laboratory studies facilitates examination of the role of sociality upon virulence. Therefore, examination of strepsipteran associated virulence among fruit fly adults, non-social hymenopterans and hemimetobolans is necessary. In addition, the costs of parasitism by other parasite taxa which infect both social and non-social hosts should be examined; a candidate taxa are tlyenchid nematodes which infect both solitary (Jaenike and Perlman 2002) and social insects (Poinar 2003). The effect that host ecology has upon parasite life history (Salt 1941, Harvey et al. 2000, Harvey and Strand 2002) is understudied in comparison to parasite mediated changes in host life history (Moret and Schmid-Hempel 2004). The social insect colony represents a homeostatic environment surrounding the habitat of the parasite (colony member), and a concentrated examination of virulence experienced by social and non-social hosts will allow an elucidation of the role that host ecology, and sociality in particular, has upon parasite life history.

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