Parasite manipulation of insect reproduction: who benefits?

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SUMMARY

Host fertility is often curtailed as a result of parasitic infection. The hypothesis that this may confer an adaptive advantage upon the symbionts if nutrients are directed from reproduction and made available for host/parasite maintenance is explored. The suggestion is made that an understanding of the mechanisms underlying the pathophysiology of fecundity reduction may shed light upon the evolutionary implications of this strategy for both parasite and host. To illustrate this the down-regulation of egg production is explored with reference to a particular model system, the association between metacestodes of the rat tapeworm, *Hymenolepis diminuta* and the mealworm beetle, *Tenebrio molitor*. Several aspects of host reproductive behaviour and physiology are affected by infection in this association, including vitellogenesis. Metacestodes directly inhibit the fat body synthesis of vitellogenin in a stage-specific, density-dependent manner. This inhibition is likely to be orchestrated by a modulator molecule, produced by the parasite. In the ovarian follicles, juvenile hormone III binding to a specific follicular membrane-binding protein is inhibited in infected beetles, resulting in the down-regulation of a cascade of events which enables vitellogenin to pass into the developing oocyte. Data to support the proposed existence of a parasite-induced antigonadotrophin, of host origin, are discussed. Evidence that similar mechanisms operate in *Plasmodium*-infected anopheline mosquitoes and *Onchocerca*-infected blackflies is presented in support of the possibility that a parasite-induced reduction in host reproductive fitness is an adaptive strategy and an assessment of who is manipulating whom is made.

Key words: *Hymenolepis diminuta*, *Tenebrio molitor*, host reproductive fitness, parasite virulence, insect vitellogenesis, juvenile hormone.

INTRODUCTION

The current balance between parasites and hosts in the evolutionary arms race can be assessed by examining the fitness of both participants in the relationship, measured in terms of transmission success of the parasite and reproductive success of the host. The interests of opposing organisms may not, however, be entirely contradictory. Trade-offs can exist between virulence and parasite fitness (Anderson & May, 1982; Ewald, 1995) because, in many situations, good health of the host can be a component of parasite fitness (Combes, 1997). Parasite life history strategy has been identified as a determining factor governing the degree of pathology induced in the host. For example, vertically transmitted parasites are less likely to reduce host reproductive success than those transmitted horizontally as host and parasite interests tend to converge (Dawkins, 1990). Unfortunately, few studies have been undertaken to test this hypothesis (but see Agnew & Koella, 1997 and Koella, Agnew & Michalakis in this volume).

An equilibrium may develop between costs to the host and benefits to the parasite that minimises immediate pathogenicity. However, maintenance of a favourable parasite environment may incur long-term costs to the host such as decreased life-span and/or reduced fertility. These long-term effects will not decrease parasite fitness if transmission occurs on or before host death. However, both will contribute to a loss of host fitness as individuals with a longer reproductive life span may have greater opportunity to contribute to the next generation. Although individual parasites could benefit by reducing host fecundity, loss of host reproductive potential may affect parasite populations in negative ways. Dobson (1988) suggested that parasitic castration is unlikely to be maintained in parasites with direct life cycles because it causes destabilization of host populations, and Combes (1997) pointed out that initiating a sharp decline in host populations could be hazardous to parasite population stability. The regulatory effects of parasites on host populations are, however, well recognized, and it is likely that the aggregated nature of parasitic infections attenuates the consequences of infection in all but epidemic situations.

As predicted, examples of parasites that actually castrate their insect hosts are rare but can be found amongst the parasitoids that attack lepidopteran eggs and larvae (Brown & Read, 1997). Complete suppression of egg development has also been reported in mermithid infections of locusts (Gordon, 1981) and strepsipteran infections in wasps (see Hurd & Webb, 1997). In contrast, some lesser disruption of host reproductive capability is widespread and can occur as a consequence of alteration of host mor-

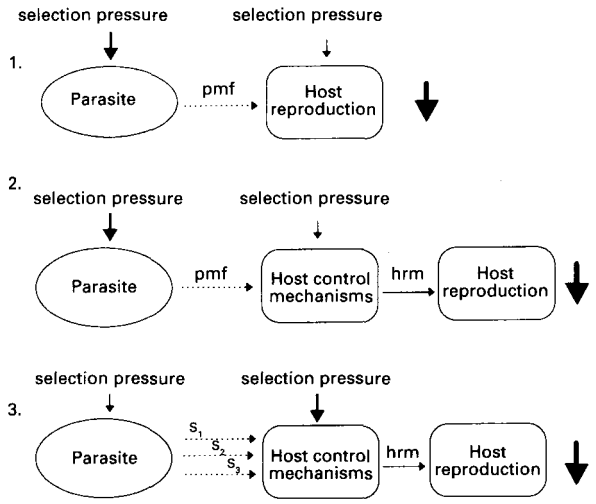

Fig. 1. Possible pathways for the operation of parasite-induced curtailment of host reproduction. S1, S2, and S3 represent pathology caused by infection, e.g. parasite toxins, nutrient deprivation, mechanical injury or stimulation of host immune response. hrm, host regulatory molecule; pmf, parasite modulatory factor.

phology, reproductive behaviour and/or physiology (previously reviewed in Hurd, 1990; 1993).

The fact that this curtailment of host reproduction has arisen many times in associations as diverse as microsporidian infections of coleopterans and mermithid infections of orthopterans (Hurd, 1990) is suggestive of an adaptive strategy (Poulin, 1995). The adaptive nature of changes in host behaviour has been rigorously examined (Moore & Gotelli, 1990; Poulin, 1994) and these analyses are of value in a consideration of the nature of parasite-induced fertility reduction. Poulin (1995) examined the nature of parasite-induced behavioural changes, defining an adaptation as a genetically determined trait that confers a selective advantage upon the organism and thus spreads in the population.

In female insects, egg production is generally very costly in terms of resource requirement, yet not essential to the survival of the individual female. It could be argued that the strategy of diverting resources away from reproductive effort would benefit the parasite by providing a richer environment, thus conferring a selective advantage upon the parasite. However, hosts are under selective pressure to compensate for the presence of parasites; if they cannot eliminate them then they must tolerate them. The host may alleviate the nutritional stress imposed by a parasitic infection and increase the chance of survival by down-regulating reproductive fitness (Forbes, 1993). This strategy could provide a selective advantage to the host if limited reproduction still occurred or if egg production eventually recommenced/recovered when parasites were eliminated. Although we can deduce that reproductive curtailment may be advantageous to both participants in the symbiosis, it is difficult to ascertain where the selective pressure is being imposed, i.e. which partner is driving this response. Who is manipulating whom?

One prerequisite for the categorization of parasite-induced fecundity reduction as an adaptive strategy is the demonstration that this response is not a by-product of other aspects of pathology (Dawkins, 1990; Hurd & Webb, 1997). If fecundity reduction is a parasite adaptation, the response should be either the result of the direct action of a parasite-derived 'modulator molecule' or a response to a parasite signal that directly initiates existing regulatory pathways. If fecundity reduction is a host adaptation, a host-derived molecule will be produced that down-regulates reproduction in direct response to the presence of the parasite. Alternatively a host-regulator molecule may not be produced solely in response to a parasitic infection, but could be a more general response to adverse conditions. Many stressors are known to disrupt physiological balances. It is feasible that some parasites induce fecundity reduction via control pathways that are not specific for parasitic infection but are also invoked in other stress situations (Fig. 1).

In associations where infection results in direct mechanical damage to gonadal tissue, or associated structures, loss of reproductive success is clearly not the result of resource investment on the part of the parasite or manipulative effort (Hurd, 1990; Poulin, 1994). Such situations are, however, rare (Brown & Read, 1997). Most responses are of a humoral nature and have been described as chemical castration (Cheng, Sullivan & Harris, 1973). Likewise, fecundity reduction cannot always be attributed to direct competition between parasite and host for nutrients (Hurd & Webb, 1997). Reproduction is usually affected early in infection when parasite biomass is small, or is not density-dependent (but see Polak, 1996). In many associations it seems likely that more refined mechanisms exist, and that reproductive physiology and behaviour may be modulated humorally via regulator molecules of host or parasite origin.

Insect reproduction is controlled by an array of neuropeptides and steroid-like hormones. As yet, no specific regulatory factor has been associated with parasitic infection and so few clues are available to identify the source of putative regulatory molecules. Parasite-derived manipulation factors could operate by interfering with intrinsic host signals or by novel mechanisms which disrupt host reproduction directly. Alternatively, parasite presence could stimulate the host to secrete specific or non-specific down-regulatory neuroendocrine signals, possibly via the host immune/neuroendocrine axes, as has recently been demonstrated in schistosome-infected snails (de Jong-Brink *et al.* 1997) (Fig. 1).

One way forward in the search for an understanding of the evolutionary status of the effect of

Table 1. Summary of the effect of metacestodes of *Hymenolepis diminuta* on the physiology of female *Tenebrio molitor*. Most effects have only been examined on certain days post-infection.

Effect on host	Timing	Reference
Ovary		
Juvenile hormone binding significantly inhibited	Greatly diminished on day 3 and 6, recovered by day 15	Webb & Hurd, 1995*b*
Na^+K^+ATPase reduced	Reduced on day 3, recovered by day 15	Webb *et al.* 1997
Patency reduced	Observed on day 3 and 9 recovered by day 15	Hurd & Arme, 1987 Webb *et al.* 1997
Vitellin content reduced	Seen on day 3 onwards, differences declines with age of infection	Hurd & Arme, 1986 Webb & Hurd, 1995*a*
Follicles resorbing	From day 9	Hurd & Arme, 1987*b*
Fat body		
Vitellogenin synthesis reduced	Significant decrease seen on days 3–30	Hurd & Arme,1986 Webb & Hurd, 1996
Glycogen content decreased	Seen from day 5	Kearns *et al.* 1994
Haemolymph		
Vitellogenin significantly increased	First seen on day 12	Hurd & Arme, 1984*a*
Trehalose significantly elevated	On day 15	Kearns *et al.* 1994
Sex pheromones		
Control male response to infected female pheromones	Significantly reduced on day 3 and 4	Hurd & Parry, 1991

parasites on host reproductive fitness is to examine the molecular mechanism(s) underlying these changes, and to identify the nature and source of any modulating factors. I have chosen to focus the remaining discussion on one particular model system that is being studied with this aim in mind; namely infection of the coleopteran, *Tenebrio molitor* by cysticercoids of the rat tapeworm, *Hymenolepis diminuta*.

THE RAT TAPEWORM/INSECT INTERMEDIATE HOST MODEL

Hymenolepis diminuta onchospheres are released from the protective membranes of the 'egg' by a combination of mechanical and enzymatic action and hatch in the gut of an insect intermediate host such as *Tenebrio molitor* (Coleoptera). The activated hexacanth larvae burrow through the midgut wall to the haemocoel (Lethbridge, 1980). During a period of approximately 10 days they grow rapidly, passing through several stages to become mature cysticercoids, infective to the definitive host (Hurd & Arme 1987*a*).

Infected beetles exhibit several behavioural and physiological changes, many of which are parasite-stage specific (Table 1). The reproductive fitness of female beetles is depressed such that egg laying is retarded and egg viability diminished. By 15 days post-infection (PI), when mature metacestodes are present, a large proportion of ovarian terminal follicles are being resorbed.

The yolk protein, vitellin (Vn), constitutes around 90% of the total soluble protein present in the eggs of *T. molitor* (Harnish & White, 1982). In common with most adult female insects, the precursor, vitellogenin (Vg) is synthesized in an extra-ovarian tissue, the fat body. Following export to the haemolymph, Vg is accumulated in developing oocytes by a process of receptor-mediated endocytosis and deposited in a crystalline form as Vn. Several aspects of this process of vitellogenesis are affected by developing metacestodes.

Events associated with the inhibition of egg production have been investigated in some detail and have been summarized recently (Hurd & Webb, 1997). Our latest findings support the view that different mechanisms may be involved in down-regulation of events in the fat body and ovary, and that modulatory factors of parasite origin may play a greater, or more direct, role in the former. Here I shall use these findings to attempt to interpret the contribution made by each partner to initiating host fecundity reduction.

EVENTS IN THE FAT BODY OF INFECTED BEETLES

Synthesis of Vg is significantly reduced throughout infection, production declining by up to 75% on day 24 PI. If normal fat bodies are cultured for 4 h with live metacestodes, Vg synthesis is inhibited by stages II to III metacestodes, but later stages of parasite development and heat killed parasites have no effect (Webb & Hurd, 1996). Acid extracts, taken from stage I and II parasites grown either *in vivo* or *in vitro*, also significantly reduced Vg synthesis by cultured fat bodies. Similar extracts from stage IV

and V larvae were not effective (Webb, Major, Ryan & Hurd, unpublished). These data generated the hypothesis that down-regulation of Vg synthesis is induced directly by a parasite-derived factor.

The site of synthesis of the active component(s) from the acid extract is unknown. Although it has not been fully characterized, the properties of this factor include heat stability, pronase sensitivity and solubility in aqueous but not methanolic media (Webb, Major & Hurd, unpublished). It is a priority to isolate and characterize this molecule as knowledge of its structure may provide clues to the exact mode of functioning.

Results from our investigations of the effect of the parasite upon the initial phase of vitellogenesis suggest that a regulator molecule of parasite origin, may be involved. It may act on fat body trophocytes, either by directly down-regulating Vg synthesis (Fig. 1.1), or indirectly, by stimulating the production of a host-derived inhibitor molecule in the fat body Fig. 1.2). Medium in which fat bodies from infected insects had previously been cultured was shown to inhibit synthesis (Major, Webb & Hurd, unpublished), suggesting the latter mechanism may be operating. Because many details of the complex nature of the molecular control of insect Vg synthesis remain unknown, it is difficult interpret these findings.

Data generated from examining fat bodies that have been exposed to developing parasites *in vivo* appear to be in conflict with those produced by short-term incubation with live parasites or parasite extracts. *In vivo* the effect is long lasting, being evident when both early and late stage parasites are present, whereas, *in vivo* inhibition is stage specific. Two possible explanations present themselves: the modification induced by stage I–II larvae may be permanent or, if transient, we may eventually be observing the effects of a negative feedback system associated with the accumulation of high titres of Vg in the haemolymph of beetles with late stage infections (Hurd & Arme, 1984*a*) as outlined below (see also Table 1).

EVENTS IN THE OVARY

T. molitor ovarian follicles of similar size normally contain the same amount of vitellin. However, in infected females, vitellin content is significantly reduced. For example, follicles of 400–600 μm from females 3 days post-infection exhibit a 50·2% lower Vn content compared with follicles from same age non-infected beetles (Webb & Hurd, 1995*a*). By day 12 PI, Vg titres in haemolymph are significantly elevated, despite the reduction in fat body synthesis. This probably results from the inhibition of Vg sequestration in terminal follicles and the lack of uptake by resorbing follicles. Haemolymph from infected female beetles, injected into non-infected recipients, was shown to simulate these effects. We demonstrated (Major, Webb & Hurd, 1997) the presence of a circulating molecule(s) that was stage specific, only being present/effective when metacestodes of stage I to IV were present in the donor female beetles. Although male beetles are also competent hosts for *H. diminuta*, haemolymph from males was unable to induce the effect in female recipients. Thus this putative antigonadotrophin cannot be derived directly from the parasite, but its production must be induced in female hosts as part of a response mechanism to the parasite (Fig. 1·3).

We feel that it is unlikely that the secretion of an antigonadotrophin would occur specifically in response to *H. diminuta* infections, but that the parasite may have evoked a non-specific stress response. However, to date, we have failed to stimulate a similar reduction in follicle Vn content and concomitant rise in haemolymph Vg in beetles stressed in other ways. There may be some parallels here with the mechanism underlying parasitic castration of snails by *Trichobilharzia ocellata* (de Jong Brink, 1995). Cercariae production is associated with the secretion of a host-derived antigonadotrophin, schistosomin, synthesised in components of the snail defence system. Schistosomin also appears to be released in short-term stress situations such as disturbance before or during egg-laying.

ENDOCRINE REGULATION OF VITELLOGENESIS

Juvenile hormone (JH), a sesquiterpenoid synthesised in the corpora allata, plays a pivotal role in the regulation of vitellogenesis in most insects (Wyatt & Davey, 1996). It functions as both a primer and regulator of protein synthesis and uptake. At the molecular level, JH has pleiotrophic actions. In the fat body of the locust it appears to act intracellularly in the nucleus, like a classic steroid/thyroid hormone, to promote gene expression (Braun *et al.*, 1995). Wyatt & Davey (1996) present evidence supporting a speculative model for JH action in the fat body nucleus via a two-step mechanism. JH is transported through the cytoplasm bound to a carrier protein to the receptor complex of an immediate response-gene A. Gene A encodes a factor required for the activation of the delayed-response gene B. Transcription is regulated by the activation of gene B. They suggest that translation efficiency of the Vg message is modulated by a neuropeptidergic brain factor and by adipokinetic hormone (Fig. 2). Adipokinetic hormone has been reported to inhibit the synthesis of Vg in *Locusta migratoria* (Glinka, Kleiman & Wyatt, 1995).

We have been unable to demonstrate any JH uptake or synthesis of JH or JH-degrading enzymes in metacestodes (Webb, Major, Borst & Hurd,

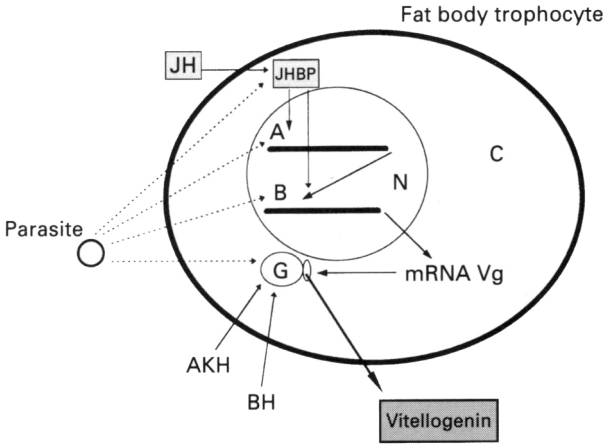

Fig. 2. Possible pathways for the action of a metacestode-derived modulator molecule based on a speculative model for the regulation of vitellogenesis in locust fat body produced by Wyatt & Davey (1996). JH is postulated as entering the cell nucleus, after diffusing through the plasma membrane in the manner of a steroid hormone. It then binds to response elements (on gene A), activating transcription of this gene. The protein produced then, together with JH again, bring about expression of gene B, which is responsible for Vg synthesis. A, Gene target of JH; B, Vg gene; G, Golgi apparatus; N, nucleus, C, cytoplasm, AKH, adipokinetic hormone; BH, brain hormone. The dotted lines represent possible points of interaction between the parasite and Vg synthesis.

unpublished) thus, it is unlikely that the parasite affects the concentration of JH that may be present in control fat bodies in our bioassay. Possibly *H. diminuta* is down-regulating Vg synthesis by affecting translation efficiency of the Vg message. The parasite may prove a useful tool with which to assist in the elucidation of vitellogenesis control mechanisms, particularly if parasite extracts are also active in other insects.

In the ovary of certain insects, JH action shows similarity to polypeptides or catecholamines in that it appears to bind to plasma membrane receptors. It operates at the membrane level in the ovarian follicular cells of *Rhodnius prolixus*, *Locusta migratoria*, and *T. molitor* by the generation of intracellular secondary messengers (Wyatt & Davey, 1996).

Developing oocytes accumulate yolk protein precursor, vitellogenin (Vg), by the process of receptor mediated microendocytosis. Synthesis of Vg receptors is regulated by juvenile hormone (JH). Vg gains access to the surface of the developing oocyte via the spaces that develop between the follicular epithelial cells that surround the developing oocytes. The development of these spaces, termed patency, is also controlled by JH. Studies of *R. prolixus*, *L. migratoria* and *T. molitor* show that juvenoids act on the follicle cells via a protein kinase C-dependent cascade to activate a JH-sensitive Na^+/K^+ ATPase. The resultant changes in ionic balance causes the follicle cells to shrink and patency develops (reviewed in Wyatt & Davey, 1996).

Specific, saturable binding of JH to the cell membrane has been demonstrated in *R. prolixus*, *L. migratoria*, and *T. molitor*. Sevala, Davey & Prestwich (1995) have partially purified a 35 kDa JH binding protein from locusts. We have shown that JH binding to cytosolic and nuclear fractions of *T. molitor* is not affected by infection with *H. diminuta*; however, binding to the membrane fraction is significantly reduced on day 3 and day 6 PI (Webb & Hurd, 1995*b*). Binding is restored to normal by day 15 PI, suggesting that parasite-induced regulation of vitellogenesis is stage specific and associated with early infections, when maturing parasites are present. Scatchard analysis revealed the presence of at least two binding sites. Although B_{max} values remained unchanged in follicles from infected females, K_d values of the higher affinity site were increased almost 5-fold. Webb & Hurd (1995*b*) concluded that these results suggest the presence of an infection-related competitive binding inhibitor rather than a reduction in the number of binding sites. If infected beetles contain a factor which competes for a JH receptor, we could expect events purported to occur down-stream from this ligand binding to be affected by infection. The model developed by Davey and colleagues (see Wyatt & Davey, 1996) included activation of a JH-sensitive Na^+/K^+ ATPase and the development of patency as two such events. A comparison of these events in infected and control beetles has therefore been made.

Patency is JH dependent in *T. molitor* (Webb *et al.* 1997). The addition of JH III to incubated ovaries elevated the patency index to a maximum at concentrations of 50 nM, a value that approximates to a physiological concentration. Patency index was significantly reduced in follicles from 3 day-old infected females. Incubation with 50 nM JH increased the patency index but did not restore it to the equivalent level of non-infected, JH incubated follicles. Down-regulation of patency appears to be reversible because prolonged washing of ovaries restored the patency index to the level of ovaries from non-infected females. (Webb *et al.* 1997).

Follicle cell ATPase from *T. molitor* possesses similar properties to ATPase from *R. prolixus* (Ilenchuk & Davey, 1982). Activity was also reduced by infection in a stage-specific manner and restored by the time that metacestodes were mature (Webb *et al.* 1997).

Our work on *T. molitor* ovarian follicles supports the hypothesis that an antigonadotrophin is active in infected beetles at a time when metacestodes are undergoing rapid growth and development. It is likely that it is this factor that we are able to transfer from infected donors to non-infected recipients. By competing for, or blocking, JH-specific binding sites

on the follicular epithelium, the factor reduces the development of patency, thereby restricting the access of vitellogenin to the oolemma. Vitellin accumulation is therefore retarded relative to follicles of the same size from normal females. It is possible that this antigonadotrophin is also responsible for the increase in resorbed follicles evident later in infection (Hurd & Arme, 1987*b*).

Many insects adjust egg production to environmental conditions, and evidence suggests that the initial response occurs in the ovary, where yolk protein uptake is inhibited (Bownes & Reid, 1990). If this is the major control point, it is surprising that so little attention has been focused upon identifying the signals involved. In flies, hormones produced by the midgut have been implicated in indirect control (e.g. Quin, Yin & Stoffolano, 1995) as has a neuropeptide from abdominal neurosecretory organs of *L. migratoria* (Davey, Sevala & Gordon, 1993). It is, therefore, possible that metacestode parasitization directly or indirectly initiates a hitherto unidentified, intrinsic regulatory mechanism in female *Tenebrio*. Whether this is a by-product of infection or a specific adaptive response on behalf of the host remains to be clarified.

EVALUATION OF THE ADAPTIVE NATURE OF CURTAILMENT OF HOST REPRODUCTION

Circulating factors appear to mediate, via endocrine control mechanisms, the down-regulation of vitellogenesis seen in *T. molitor* infected by *H. diminuta*. Although we are unable to determine unequivocally whether this intervention is initiated as a result of the 'extended phenotype' of the parasite or a 'boring by-product' of infection (Dawkins, 1990), evidence from studies of the fat body strongly support the former. However, host involvement also seems to be indicated when events in the ovary are examined. The interaction is clearly complex and thus fulfils one of the criteria identified as indicative of an adaptive change. Three additional indicators were identified by Poulin (1995), namely purposiveness of design, convergence and fitness effects. Our insight into the precise orchestration of reproductive curtailment is not yet sufficient to enable us to evaluate how well the manipulation is fitted to increase host and/or parasite fitness, but the other two criteria will be applied to the model.

The observation that parasites from diverse lineages so often have a negative impact on insect reproduction is suggestive of convergence (Hurd, 1990, 1993). Unfortunately, the underlying mechanisms have been investigated in very few of these associations, although where they have remarkably similar pathways have been identified. Mermithid infection appears to disrupt locust vitellogenesis in a manner reminiscent of the model described here (Gordon, Webster & Hislop., 1973; Condon & Gordon, 1977). Another nematode, *Onchocerca lienalis*, inhibits both fat body synthesis of vitellogenin and accumulation of the yolk protein in the ovaries of the blackfly, *Simulium ornatum* (Renshaw & Hurd, 1994). In both blackflies and an anopheline mosquito acting as a vector for *Plasmodium yoelii nigeriensis*, inhibition of Vg sequestration is also associated with an accumulation of Vg in the haemolymph (Renshaw & Hurd, 1994, Jehan & Hurd, unpublished).

Several authors have suggested that JH action is disturbed in parasitized insects (e.g. Fisher & Sandborn, 1964; Condon & Gordon, 1977; Röseler & Röseler, 1973; Strambi, Strambi & Augier, 1982) largely as a result of a reduction in circulating titres. This is not the case in the *H. diminuta*/*T. molitor* model, where tissue response rather than circulating titres are affected (Hurd, Strambi & Beckage, 1990). Much of the work on other systems is inconclusive or even more speculative and it would be helpful if some of these models were revisited using currently available techniques.

H. diminuta infects several other tenebrionids, including *Tribolium* spp., which are widely used as laboratory models (Yan, Stevens & Schall, 1994). Although reproductive physiology has not been examined in these associations, the negative impact of *H. diminuta* infections on *T. confusum* reproduction has been described (e.g. Keymer, 1980; Maema, 1986). Maema (1986) found that, provided parasite burden was sufficiently high, fecundity was significantly reduced early in infection, and both she and Keymer (1980) demonstrated an exponential relationship between parasite biomass and fecundity reduction.

Fig. 3 illustrates how fecundity reduction could increase either host or parasite fitness if a reduction in egg production resulted in a shift of nutrient resources from egg production to host reserves and to the parasite. It is axiomatic that some of these resources are utilized by the developing metacestodes (Arme, 1988; Rosen & Uglem, 1988; Pappas & Durka, 1993) and thus could contribute to the likelihood of parasite transmission. Parasite transmission opportunities will also be enhanced if host longevity is increased.

A full analysis of energy budgets in non-infected and infected females has not been performed but it seems unlikely that resources released by lowered egg production completely compensate for nutrients removed by the parasites, as both carbohydrate and amino acid reserves in female *Tenebrio* are eventually depleted by infection (Kearns, Hurd & Pullin, 1994; Hurd & Arme, 1984*b*). In addition, Blackburn, Modha & Novak (1995) used ^{31}P NMR studies to reveal increases in the phosphorylation potential of infected beetles that were more pronounced in females. However, it could be envisaged that manipulation of reserves, at a time when parasites were

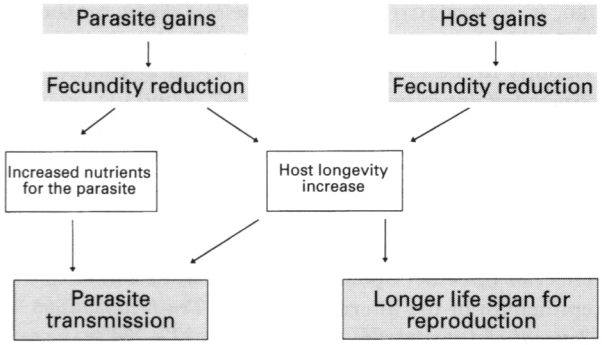

Fig. 3. The adaptive significance of fecundity reduction.

growing rapidly, could reduce the stress imposed by infection and enhance the chances of host survival (discussed further in Hurd & Webb, 1997).

We now have evidence that, in ideal laboratory conditions, infected female beetles do survive significantly longer that non-infected counterparts (Major & Hurd, unpublished). Lifetime reproductive output of infected females has not been examined but egg output eventually recovers (Hurd & Arme, 1986) from the effect of early infection. It is therefore possible that parasite-induced fecundity reduction may confer fitness benefits on at least one of the symbionts and both scenarios illustrated in Fig. 3 could occur in this association.

CONCLUSION

Study of the *H. diminuta*/*T. molitor* model illustrates the fact that physiological and biochemical studies of host–parasite interactions can shed some light on the evolutionary history of these relationships.

It appears that signals from both host and parasite may be modulating host reproductive output. Evidence in support to the existence of a parasite-derived factor that inhibits Vg synthesis is accumulating and the nature of this molecule is being investigated. In contrast, a factor of host origin may be operating at the level of the ovary. Several questions arise concerning this putative, parasite-induced, antigonadotrophin. First, is it synthesized by the host as part of the normal repertoire of control molecules or specifically in response to signals from the parasite? Secondly, what is the nature and source of the molecule? And lastly, does it compete directly for the JH binding site or does it inhibit JH binding indirectly, from an adjacent receptor site?

In order to respond to presence of an endoparasite, the host must recognize it. It appears that haemocytes of *T. molitor* recognise *H. diminuta* metacestodes, even though the beetle cannot mount an effective immune response (Richards & Arme, 1985). It is, therefore, likely that the parasite tegument or excretory/secretory products are detected. Tentative links are now being established between endocrine, nervous and defence systems in invertebrates. If this is a three-way signalling system it could provide the means for the insect host to initiate a co-ordinated response to an invader, as has been suggested for snails infected with trematodes (de Jong-Brink, 1995). Parasites that regulate host reproduction could thus be making use of existing stress response systems or specific pathways may have evolved in the host in response to infection.

The particular model that has been explored in this review provides some evidence that curtailment of host reproduction fulfils the criteria suggestive of an adaptive change in physiology, of benefit to the parasite, but the situation is by no means conclusive.

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