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Direct and indirect pathways of fitness-impact in a protozoan-infected kissing bug

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Abstract. Parasites can reduce host fitness through short-term mortality, complete or partial castration, or slight reductions in host fecundity. Hosts may reduce reproductive effort as an adaptive strategy to tolerate parasitism. However, host fitness reduction may be unrelated to host adaptation but represent a pathological side-effect of infection. The present study evaluates experimentally the direct and indirect impact of the protozoan parasite *Trypanosoma cruzi* on the investment of female kissing bugs in reproductive tissue. The presence of the parasite decreases gonad weight but this effect disappears when body size is included as covariate. To examine in more detail the set of causal relationships involved, a structural equation modelling analysis is performed using body size, moulting time and nutrition as predictor variables on gonad weight in the presence and absence of the protozoan. The results obtained indicate that, irrespective of the pathway and status of infection, female kissing bugs showing a slow development tend to have lighter gonads. On the other hand, the importance of blood ingestion for gonad weight is dependent on body size and contingent on the status of infection. Uninfected individuals tend to invest more in reproductive tissue when ingesting more blood during their ontogeny, and the opposite situation is observed for infected insects. These results indicate that gonad weight reduction in *T. cruzi*-infected *Mepraia spinolai* (Porter, 1934) is a consequence of nutrition curtailment and body size reduction rather than an adaptive strategy to cope with infection.

Key words. Gonad weight, *Mepraia spinolai*, Reduviidae, reproductive investment, Triatominae, *Trypanosoma cruzi*, vector-parasite interaction.

Introduction

Parasites can reduce host fitness through short-term mortality, complete or partial castration, or through slight reductions in host fecundity (Minchella, 1985; Ballabeni, 1995; Poulin, 1998). Although the effects of parasitism are often observed, there is as yet little understanding of the mechanisms underlying host fecundity reduction (Moore, 2002). Several studies interpret the reduction in host reproductive success as an adaptive strategy to tolerate parasitism (Poulin, 1998), assuming that organisms have limited resources for reproduction, and that resource allocation to defence, growth and maintenance compromises resource allocation to reproduction (Forbes, 1993; Perrin *et al.*, 1996). On the other hand, host fitness reduction is interpreted as a simple pathological side-effect of infection unrelated to host adaptation (Poulin, 2000; Wilson, 2000). These general interpretations are difficult to test, although they are useful in separating ultimate from proximate and physiological explanations for host fitness reduction. Most studies assume that adaptive responses occur only when parasites have a direct impact on host reproduction, restricting responses that involve indirect changes in fitness-related traits to non-adaptive pathological side-effects.

In insects, most studies examining the influence of parasitism on host fitness focus only on the direct effects, ignoring the potential contribution of indirect effects to host fitness (Poulin, 1998; Moore, 2002). Direct effects measure the relationship between an independent and a dependent variable in

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the absence of any mediating effects. By contrast, indirect effects measure the relationship between variables when a mediating variable intervenes. This situation is unfortunate because indirect effects may compensate or even overcompensate the direct negative fitness-impact of parasitism (Minchella, 1985). The present study focuses on a trypanosome–triatomine system that consists of the protozoan parasite *Trypanosoma cruzi* and its vector *Mepraia spinolai* (Hemiptera: Reduviidae). Whether parasitism affects host investment in reproductive tissue and the pathway of causation involved is investigated. More specifically, the specific questions addressed are: does *T. cruzi* infection reduce gonad weight in *M. spinolai* females? If reduction in reproductive tissue investment occurs, what is the importance of body size in this reduction? To what extent is gonad weight related to developmental time and nutrition, and what are the direct and indirect pathways of causation? To examine the complex set of causal relationships among variables, a structural equation modelling (SEM) analysis on kissing bugs in the presence and absence of *T. cruzi* was undertaken.

Trypanosoma cruzi is the causative agent of Chagas disease. This heteroxenous trypanosomatid possesses a life cycle that involves several morphologically distinct stages. Once inside the insect vector, the trypanosomatid multiplies and differentiates in the digestive tract. Infection of definitive mammal hosts occurs by contamination with the infectious metacyclic trypomastigote stage of the flagellate (Kollien & Schaub, 1997, 2000). The kissing bug *M. spinolai* (Porter, 1934) is one of the triatomine species responsible for *T. cruzi* transmission in arid and semiarid Chile (Lent *et al.*, 1994). This strictly haematophagous and diurnal vector species is distributed over the range 18–34°S and its main habitat includes bird nests, rock crevices and caves (Lent & Wygodzinsky, 1979; Botto-Mahan *et al.*, 2005a). *Mepraia spinolai* requires blood of vertebrates to complete its life cycle (Sagua *et al.*, 2000; Canals *et al.*, 2001). The development of this hemimetabolous insect includes an egg, five nymphal instars and the adult. Often, one full engorgement is sufficient for moulting from one nymphal instar to the next (Kollien & Schaub, 2000). No information has been published about ovarian development in *M. spinolai*. However, a report on *Triatoma infestans* (Reduviidae, Triatominae) indicates that, by the seventh day after imaginal moulting, adult females show vitellogenic oocytes, by the 13th day, coriogenesis and ovulation begin and, by the 21st day, the eggs are fully developed (Asin & Crocco, 1992).

Materials and methods

Control and infected female bugs

Mepraia spinolai eggs were obtained from laboratory mating between adults collected at Las Chinchillas National Reserve (31°30'S, 71°06'W, IV Region, Chile; Botto-Mahan *et al.*, 2002). Eggs were removed from the mating jars daily, cleaned and placed in sterile plastic containers. Once the first-instar nymphs emerged, a random assignment to infected (fed on *T. cruzi*-infected hosts) or control (fed on uninfected hosts) was performed. Each nymph was individually housed in a 3.2 × 3.6 cm clear plastic compartment of an 18-compartment box (11.4 × 20.5 cm). Each compartment was provided with sandy bottom and a folded piece of paper as refuge. All insects were reared under optimal growing conditions in a climatic chamber at 26 °C, 65–70% relative humidity and under an LD 14 : 10 h photoperiod (Ehrenfeld *et al.*, 1998). Nymphs were infected at the first feed, 3–4 weeks after eclosion, using *T. cruzi*-infected laboratory mice (C_3H mouse strain, Central Breeding Ground, Facultad de Medicina, Universidad de Chile, Santiago, Chile). The *T. cruzi* strain used to infect was isolated in May 2002 from *M. spinolai* collected at the field (Las Chinchillas National Reserve, Chile; Botto-Mahan *et al.*, 2005b). Trypanosomes in faeces and urine of field-captured insects were used to infect mice by intraperitoneal inoculation. The *T. cruzi* strain was maintained by cyclical transmission across mouse generations (10^4 blood trypomastigotes; Wallace *et al.*, 2001). Only infected mice with increasing parasitaemia were used for feeding purposes. Control nymphs were fed 3–4 weeks after eclosion on uninfected laboratory mice. After each moult, infected and control nymphs were starved for 3 weeks and then fed on infected and uninfected mice, respectively, until adult emergence. Nymphal weight was recorded before and after feeding (to a precision of 0.1 mg). Moulting was recorded on a daily basis and, once adults emerged, sex was determined according to sexually dimorphic characters (Lent & Wygodzinsky, 1979). To ensure that infected nymphs were truly infected, the presence of *T. cruzi* in faecal samples of fifth-stage nymphs was verified by light microscopy (Nikon Diaphot-FXA, Nikon, Tokyo, Japan), compressing 5 μL of fresh faecal drops between a slide and an 18 × 18 mm cover-slip. The presence of motile parasites in 50 microscopic fields was recorded at ×400 magnification. All kissing bugs fed on infected mice showed evidence of *T. cruzi* in their faeces.

Variables measured

Infected ($n = 65$) and uninfected ($n = 109$) female nymphs reaching maturity were weighed and photographed with a digital camera (Sony Cybershot DSC-F717 5 MP, Sony Corp., Tokyo, Japan), 1 day after moulting to adult. To obtain comparable measures, females were placed on paper marked out in millimetres and photographed in a dorsal view. Measurements were obtained from digital photographs using Uthscsa ImageTool for Windows, version 2.01 (Wilcox *et al.*, 2000). The body length was measured as the distance between the head and the last segment of the abdomen. Abdomen width was measured as the widest part of the abdomen (IV–V intersegmental suture). One month after maturity, bugs were dissected to extract and weigh their fresh ovaries. Nutrition was quantified as the total weight of the blood ingested during insect ontogeny (from first- to fifth-instar nymph). Moulting time was calculated as the total number of days elapsing between the initial feeding as first-nymphal instar until adult emergence.

Statistical analysis

To examine whether gonad weight was affected by the infection status of female bugs, a one-way analysis of variance (ANOVA) with infection status as a single factor was performed. In addition, a one-way analysis of covariance (ANCOVA) with infection status as single factor and a principal component of body size as covariate was performed. The body size was estimated from the linear combination of equations for body length (mm), abdomen width (mm) and body weight (mg), which explained 91.2% of variance. A second analysis included a correlation matrix among body size, the ingested blood (mg), moulting time (days) and gonad weight (mg). Variables were checked for homogeneity of variance by using the F_{max} test. To obtain normality, variables were log-transformed when necessary (Sokal & Rohlf, 1995).

To quantify the set of causal relationships among variables and gonad weight in adult female kissing bugs, a path diagram of causal relationships was constructed and the adequacy of the hypothesized model to the real covariance structure of data was estimated by employing SEM analysis using the software EQS (Bentler, 1995). The power of SEM analysis to test evolutionary and ecological hypothesis has been stressed by several authors (Crespi & Bookstein, 1989; Mitchell, 1992; Medel, 2001). This modelling procedure is a form of path analysis that allows one to analyze more complex sets of causal relationships, including factors from multivariate principal components (Dunn *et al.*, 1993; Byrne, 1994). In this analysis, moulting time and ingested blood were used as predictor variables, a factor reflecting body size was estimated from body length and abdomen width and gonad weight was the dependent variable.

Results

Gonad weight differed between infected and uninfected female bugs (one-way ANOVA: $F_{1,169} = 10.602$, $P = 0.0014$). Gonads of infected females weighed 36.7% less than those of uninfected ones. However, this effect disappeared after including the principal component of body size as covariate in a one-way ANCOVA ($F_{1,168} = 0.335$, $P = 0.564$), indicating that body weight accounted for an important fraction of the variance in gonad weight. Table 1 summarizes the descriptive statistics of the variables measured on adult female bugs. All variables related to body size showed a reduction when insects were infected with *T. cruzi*. For example, infected females weighed 24.6% less than uninfected ones. Length and width measurements were reduced by 3.7% and 6.4%, respectively, of infected insects. The total volume of blood ingested during insect ontogeny showed a 14.9% reduction for infected female bugs on average. Finally, infected insects needed an additional 43 days to reach maturity (20.4%) on average.

Results from the correlation matrix among variables indicated that body size correlated positively and significantly with the total volume of ingested blood and gonad weight for uninfected and infected female bugs (Table 2). In turn, the ingested blood correlated positively and significantly with gonad weight only for uninfected female bugs (Table 2). Moulting time did not correlate significantly with any of the measured variables or with the body size for uninfected and infected female bugs (Table 2).

Table 1. Descriptive statistics (mean ± SE) of variables measured on adult *Mepraia spinolai* females.

Variables	Uninfected ($n = 109$)	Infected ($n = 62$)
Adult weight (mg)	175.30 ± 3.92	132.07 ± 4.46
Gonad weight (mg)	24.93 ± 1.61	15.77 ± 1.48
Body length (mm)	19.36 ± 0.09	18.65 ± 0.09
Abdomen width (mm)	7.78 ± 0.06	7.28 ± 0.07
Ingested blood (mg)	616.76 ± 13.24	524.59 ± 15.87
Moulting time (days)	210.38 ± 5.33	253.21 ± 7.52

The hypothesized set of causal relationships among variables did not differ from the covariance structure of data (Bentler–Bonett normed fit index: uninfected = 0.986, infected = 0.989; Fig. 1), which indicates that the hypothesized model was supported statistically (uninfected: $\chi^2 = 5.29$, d.f. = 2, $P = 0.07$; infected: $\chi^2 = 1.18$, d.f. = 2, $P = 0.55$). Results from SEM analysis revealed positive path coefficients between the body size factor and gonad weight for uninfected and infected female bugs (0.16 and 0.63, respectively). However, this coefficient was statistically significant only in infected kissing bugs. The ingested blood showed a positive, but not significant, direct effect on the response variable for uninfected female insects (path coefficient = 0.42) and a negative and significant direct effect for infected insects (path coefficient = −0.28). In the two groups, the ingested blood had a positive and significant direct influence on body size. Moulting time, in turn, had a negative and not significant direct effect on gonad weight. Similarly, moulting time influenced negatively the body size in both groups but this effect was only significant for uninfected female bugs (Fig. 1). Regarding indirect effects, the ingested blood indirectly affected gonad weight through body size in the two groups, although this effect was stronger in infected than uninfected insects (Table 3). Interestingly, in the infected group, the indirect effect of ingested blood on gonad weight compensated for to a large extent the negative direct effect between variables. This compensation resulted in a ten-fold lower total impact of ingested blood on gonad weight compared with uninfected bugs (Table 3). Indirect but slight effects were also detected for moulting time on gonad weight but these effects did not translate into important differences between groups. Overall, the ingested blood showed the strongest total effect on gonad weight for uninfected kissing bugs, and body size had the strongest total effect for infected ones (Table 3).

Discussion

The results obtained in the present study reveal that the investment of female *M. spinolai* in reproductive tissue is

Table 2. Correlation matrix among variables measured on uninfected (above the diagonal) and infected (below the diagonal) adult *Mepraia spinolai* females.

	Body size	Ingested blood	Moulting time	Gonad weight
Body size	—	0.431***	−0.071	0.430***
Ingested blood	0.637***	—	0.098	0.431***
Moulting time	−0.106	0.015	—	−0.070
Gonad weight	0.449***	0.130	−0.098	—

Body size corresponds to a principal component of body size calculated from adult weight, body length and abdomen width of female insects. ***$P < 0.001$. Significant correlations kept significance after Bonferroni adjustment with a tablewise α-level = 0.05. Correlations were calculated using log-transformed variables.

contingent on the presence or absence of *T. cruzi*, being higher in the absence of the parasite. However, the effect of the protozoan parasite disappears when body size is included as covariate. Hurd *et al.* (1995) suggest that female body size might affect reproduction in two ways. First, fecundity can be constrained by the number of ovarioles present in each ovary, which is often a function of body size. Second, body weight reduction may affect blood feeding and bloodmeal utilization for egg production. The results from the present study show that infected *M. spinolai* have a 1.3-fold body weight reduction compared with control individuals, and this reduction translates into an ovary size reduction as expected. The fact that infected females reach a smaller size at maturity compared with uninfected ones suggests that *T. cruzi* probably curtails essential nutrients involved in host growth (Thompson, 1983; Hurd, 1990). Like most cases of parasite–insect vector relationships (e.g. Leishmania-infected sandflies, filarial worm-infected mosquitoes, filarial nematode-infected blackflies; Hurd, 2003), trypanosomatids and their insect hosts appear to compete for metabolites and trace nutrients that are present in the ingested blood (Schaub, 1994; Kollien & Schaub, 2000). Because, in this experimental design, infected kissing bugs are exposed to *T. cruzi* infection as early as the first nymphal stage, it is highly probable that parasites and insects compete for nutrients during host development. However, this potential mechanism for host size decrease requires that infected hosts reduce energy allocation to growth and maintenance. Nonetheless, studies performed on molluscs show exactly the opposite pattern, namely an increase in host size due to infection (Minchella, 1985; Lim & Green, 1991; Ballabeni, 1995). These alterations have been interpreted as a host physiological response that diverts resources from reproduction to somatic growth

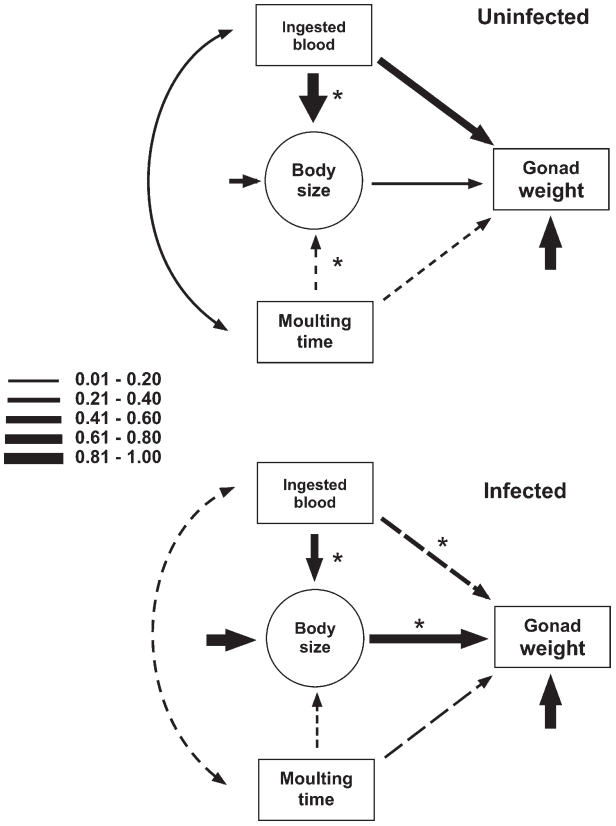

Fig. 1. Path diagrams of causal relationships among: (i) a factor of body size (calculated from body length and abdomen width); (ii) the predictor variables, ingested blood (total amount ingested through insect development) and moulting time (calculated as the time elapsed from the first instar nymph to adult); and (iii) the response variable, gonad weight (measured at maturity). Dashed lines indicate negative path coefficients. Line thickness indicates coefficient range value as shown. Asterisks indicate statistically significant coefficient ($P < 0.05$).

Table 3. Direct, indirect and total effects of predictor variables on gonad weight for uninfected and infected *Mepraia spinolai*.

	Direct effect	Indirect effect	Total effect
Uninfected			
Body size	0.159	—	0.159
Ingested blood	0.418	0.149	0.567
Moulting time	−0.068	−0.021	−0.089
Infected			
Body size	0.635	—	0.635
Ingested blood	−0.280	0.329	0.049
Moulting time	−0.121	−0.011	−0.132

The indirect effects were calculated from the product of sequential path coefficients.

and maintenance in situations of partial or complete parasite-induced castration (Ballabeni, 1995). Even though trypanosomatids do not increase growth and lifespan of their insect hosts through castration or fecundity reduction (Schaub, 1994), the possibility that host size reduction is the result of fine-tuned energy allocation processes that favor insect survival over somatic growth cannot be ruled out.

The results obtained in the present study indicate that infected insects ingest less blood during their ontogeny than control individuals. This is due mainly to a correlated reduction with body size (i.e. body weight, length and width). This finding is consistent with behavioural data on the feeding performance of *M. spinolai* that show that feeding efficiency and size of bloodmeals decrease in *T. cruzi*-infected insects (Botto-Mahan *et al.*, 2006). Interestingly, the amount of blood ingested has direct and indirect effects on the investment in reproductive tissue in female insects, and the magnitude and sign of such effects are contingent on the status of infection. For example, the reproductive investment in gonad weight in uninfected insects is dependent on the amount of blood ingested (i.e. nutrition), both directly (path coefficient = 0.418) and indirectly through an increase in body size (product of the path coefficients = 0.149). This is probably due to correlations between body size and many body organs. These results indicate that, under optimal feeding conditions, uninfected insects can express their maximum reproductive potential.

With regard to the influence of developmental time on gonad weight, the direct and indirect effects of moulting time are of a lower magnitude than nutrition and are always negative. This may arise because individuals requiring more time to reach maturity divert energy to somatic maintenance and survival instead of reproduction. When comparing these results with the network of relationships for infected insects, similarities, but also strong differences, in the magnitude and sign of direct, indirect and total effects are detected. For example, individuals having a long developmental time possess lighter gonads irrespective of their status of infection. This indicates that, at least in this system, the magnitude and sign of developmental time effects on the reproductive investment do not depend on the presence of *T. cruzi*. Unlike uninfected kissing bugs, however, the reproductive investment of infected insects is determined strongly by body size (path coefficients: uninfected = 0.159, infected = 0.635). In the same vein, there is a conspicuous change in the sign and magnitude of the direct effect of nutrition on the reproductive investment (from 0.418 to −0.280) in uninfected and infected females, respectively. This indicates that infected insects tend to invest less in reproductive tissue per unit of blood ingested during their ontogeny compared with uninfected individuals. One possible explanation for this finding is that well-fed insects may burden greater numbers of parasites, especially after long periods of starvation previous to an engorgement. It is known that *T. cruzi* burden decreases by 99.5% in the rectum of the kissing bug *T. infestans* during long periods of starvation (Kollien & Schaub, 2000), and a large blood meal would induce parasite multiplication. In the present study, it is suggested that *T. cruzi* multiplication may be triggered when infected *M. spinolai* feed on larger volumes of blood, which results in a higher parasite population in the digestive tract that is maintained at the expense of vector growing and reproduction.

In summary, gonad weight reduction in *T. cruzi*-infected *M. spinolai* appears to be a collateral consequence of nutrition curtailment and body size reduction rather than a host adaptation to survive parasitism. Our findings are relevant considering that the reproductive capacity of one species is important in its population dynamics.

Notwithstanding, the effect of *T. cruzi* on vector fecundity, in terms of quantity and quality of progeny, must be addressed to elucidate the ultimate fitness impact of this protozoan on its insect vector.

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