Modificación fenotípica inducida por parásitos:

la interacción *Mepraia spinolai – Trypanosoma cruzi*

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A los míos

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RESUMEN

En una gran variedad de hospederos se han descrito cambios fenotípicos inducidos por parásitos. Tales cambios se han interpretado como adaptativos para el parásito y/o hospedero, o simples consecuencias patológicas de la infección. En sistemas hospedero-parásito con participación de hospederos intermediarios, la evidencia empírica indica que la modificación fenotípica de tales hospederos puede favorecer la transmisión del parásito hacia hospederos depredadores definitivos mediante un incremento en la vulnerabilidad a la depredación. De este modo, la transmisión parasitaria ocurriría en estrecha relación a una interacción depredador-presa. Sin embargo, los parásitos pueden aumentar su transmisión no sólo incrementando la vulnerabilidad del hospedero intermediario a la depredación, sino también mediante un aumento en la tasa de contactos efectivos no depredatorios entre el vector y el hospedero definitivo. La transmisión mediada por vectores puede ocurrir mediante la saliva del vector al momento de alimentarse de un hospedero o mediante la deyección del vector sobre el hospedero del cual se alimenta. Al respecto, existe evidencia que apoya la idea que cambios conductuales en el proceso de alimentación inducidos por parásitos salivales favorecen la transmisión parasitaria. En esta tesis, evalúo si un parásito de transmisión por deyección induce modificaciones en el fenotipo de su insecto vector. Se examinó de qué manera modificaciones en diversos rasgos del fenotipo del vector, tales como el tiempo de desarrollo, rasgos conductuales, y tamaño corporal podrían favorecer la transmisión del parásito flagelado hacia hospederos definitivos.

El estudio se efectuó en un sistema endémico constituido por la vinchuca silvestre, *Mepraia spinolai* (Hemiptera: Reduviidae), y el parásito flagelado causante de la enfermedad de Chagas, *Trypanosoma cruzi* (Protozoa). Se siguieron cohortes de *M. spinolai* correspondientes a la primera generación en cautiverio, las cuales fueron alimentadas con roedores de laboratorio infectados con *T. cruzi* (cohortes tratamiento) y no infectados (cohortes control).

Los resultados indican que los insectos infectados presentan mayores tiempos de muda que los controles. El impacto del parásito fue dependiente del estadío considerado, siendo los tiempos de muda de los tres últimos estadíos ninfales los más afectados. Adicionalmente, *T. cruzi* disminuyó los pesos corporales de los machos y las hembras de los últimos tres estadíos. Cuando el sexo del hospedero fue considerado, las hembras se demoraron más tiempo que los machos en desarrollarse al estadío adulto. Por lo tanto, el impacto de *T. cruzi* fue dependiente del sexo. Estos resultados indican que *T. cruzi* modifica el tiempo de desarrollo de *M. spinolai*, lo cual constituye la primera evidencia de modificación fenotípica inducida por parásitos dependiente del sexo y estadío.

Los insectos vectores infectados presentan una reducción en el tiempo de localización, un aumento en el número de picadas y una disminución en la cantidad de sangre ingerida desde hospederos vertebrados. El tiempo transcurrido entre el fin de la ingesta de sangre y la deyección se redujo en presencia del parásito, sugiriendo que cambios mediados por parásitos en la conducta de alimentación y patrón de deyección de *M. spinolai* promueven la dispersión de los tripanosomátidos hacia hospederos definitivos.

Se evaluó experimentalmente la contribución separada de machos infectados y hembras infectadas en la reproducción de *M. spinolai*. El parasitismo de machos y hembras no afectó la tasa de producción de huevos, la cantidad de huevos, el porcentaje de huevos con vitelo, el porcentaje de eclosión, y el número y peso de las ninfas del primer estadío. Sin embargo, los huevos de los parentales infectados fueron más livianos que aquellos provenientes de parentales controles, indicando que el protozoo afectó la calidad más que la cantidad de los huevos. Este resultado indica que la infección de machos y hembras afecta de igual manera el desempeño reproductivo de *M. spinolai*.

El análisis e integración de la evidencia aquí presentada sugiere que, aun cuando *T. cruzi* no pareciera impactar sustancialmente en la fecundidad de *M. spinolai*, los cambios que induce en la ontogenia y conducta de alimentación del insecto debieran traducirse en un mayor potencial de transmisión de la infección hacia hospederos definitivos. Más antecedentes desde una perspectiva fisiológica son necesarios para explicar los mecanismos que subyacen a las modificaciones en los rasgos de historia de vida y conducta del insecto vector *Mepraia spinolai* inducidos por el parásito *Trypanosoma cruzi*.

ABSTRACT

Parasite-induced phenotype changes have been described in a wide range of hosts. These changes have been explained as parasite adaptations, host adaptations, or mere side-effect pathologies of parasitic infections. In host-parasite systems with intermediate and definitive hosts, empirical evidence indicates that intermediate host phenotype modifications could increase parasite transmission towards predatory definitive hosts by increasing the vulnerability of intermediate hosts to predation. In this way, parasite transmission is closely related to a predator-prey interaction. However, parasites can increase transmission not only through increases in intermediate host vulnerability, but also through increases in the rate of non-predatory effective contacts between vectors and definitive hosts. Vector-borne parasites can be salivary transmitted by vectors probing definitive hosts or by vector-infected dejections on bitten definitive hosts. Behavioral changes in the feeding process have been reported for salivary transmitted parasites that enhance infection transmission. In this study, I assess if a dejection-transmitted parasite induces alterations in the vector phenotype. I examine whether modifications in vector phenotypic traits such as developmental time, feeding behavior, and body weight influence the transmission of the parasite towards definitive hosts.

This study was carried out in a trypanosomatid-insect relationship that is endemic to Chilean arid zones. It consists on the causative agent of Chagas disease, the parasite *Trypanosoma cruzi* (Protozoa), and its wild vector *Mepraia spinolai* (Hemiptera:

Reduviidae). Laboratory cohorts of *M. spinolai* were established and fed on *T. cruzi*-infected and uninfected mice.

Results indicate that *T. cruzi*-infected bugs showed a slower moulting time than uninfected individuals. The impact of the parasite was age-dependent, as the last three moults were the most affected stages. In addition, *T. cruzi* decreased significantly the weight of males and females in the three last stages. When insect sex was taken into account, female kissingbugs took longer time than males to develop into the adult stage, which implies that the impact of *T. cruzi* is sex-dependent. These results indicate that *T. cruzi* modifies the phenotype of *M. spinolai*, and provide the first evidence for age and sex-dependent parasite-induced phenotype modification.

Infected *Mepraia spinolai* reduced the time needed for host vertebrate location, increased the number of insect biting attempts, and decreased the blood intake from the vertebrate host. The time elapsed between blood intake and dejection was reduced in the presence of the parasite, suggesting that parasite-mediated changes in the feeding behavior and dejection pattern of *M. spinolai* may promote the spread of trypanosomes toward definitive hosts.

Using an experimental design, I separated the contribution of male and female infection on kissingbug reproduction. Parasitism in males and females did not affect the egg-laying rate, egg clutch size, the percentage of eggs with vitellus, the percentage of egg hatching, and the number and weight of first instar nymphs. Eggs coming from infected parents, however, were lighter than eggs coming from uninfected bugs, indicating that the protozoan affected egg quality rather than egg quantity. This finding

suggests that *T. cruzi* inflicts a small reproductive cost to *M. spinolai* regardless of the sex that is infected.

Overall, the evidence here presented suggests that, even though *T. cruzi* has a small impact on *M. spinolai* fecundity, it modifies the ontogeny and feeding behavior of the insect vector, which should translate into a higher chance of parasite transmission towards a definitive host. More physiological evidence is necessary to understand the mechanisms underlying the life history and behavioral modifications observed in *Mepraia spinolai* insects infected with *Trypanosoma cruzi*.

1. INTRODUCCION

Un paradigma importante en biología evolutiva de interacciones hospedero-parásito es la habilidad de los parásitos para manipular el fenotipo de sus hospederos y así facilitar su propia transmisión (ver Poulin 1998a, 1998b, 2000). La modificación fenotípica inducida por parásitos ocurre cuando el valor poblacional promedio de un carácter fenotípico cambia como consecuencia de la infección parasítica. La importancia de este efecto para la población hospedera depende de la abundancia del parásito y del número promedio de parásitos por hospedero (Poulin & Thomas 1999). Existe evidencia que indica que los parásitos modifican un amplio rango de caracteres en sus hospederos. Tales aspectos incluyen modificaciones fisiológicas (Haye & Ojeda 1998; Poulin & Thomas 1999), conductuales (e.g., Moore 1983; Curtis 1987; Gotelli & Moore 1992; Hechtel *et al.* 1993; Poulin 1993; Krause & Godin 1994; Yan *et al.* 1994; Vance 1996) y morfológicas (e.g., Hechtel *et al.* 1993; LoBue & Bell 1993; Ballabeni 1995). Estos tipos de modificaciones han sido descritas para diversas asociaciones hospedero-parásito, incluyendo hospederos vertebrados e invertebrados, los cuales interactúan tanto con endo como ectoparásitos (Poulin 1994). Aun cuando este fenómeno ha sido ampliamente documentado en la literatura, el grado en el cual la modificación en el fenotipo del hospedero es adaptativa continúa siendo una controversia (Stamp 1981; Poulin 1994; Poulin & Thomas 1999; Poulin 2000; Wilson 2000).

Desde una perspectiva evolutiva, los cambios en el hospedero pueden ser adaptaciones para el propio hospedero, si la modificación conlleva una eliminación del

parásito, una mejor tolerancia al parasitismo, o una reducción en el impacto del parásito sobre su adecuación biológica (Minchella 1985; Clayton & Wolfe 1993). Por otro lado, si el cambio es adaptativo para el parásito (hipótesis de manipulación), este último debiera obtener algún tipo de beneficio tal como un aumento en su probabilidad de transmisión hacia otros hospederos (Poulin 1995) y/o su tiempo de persistencia (Brown 1999). Por último, si la modificación del fenotipo es una mera consecuencia patológica de la infección, el cambio sería no-adaptativo para ambos interactuantes (Poulin 1995, 1998b; Poulin & Thomas 1999). Un problema en los estudios realizados en esta área de investigación es el uso poco riguroso del término adaptación (Combes 1991; Poulin 1995). En general, cualquier cambio en el fenotipo del hospedero que sigue a la infección ha sido laxamente aceptado como el resultado de la selección natural actuando sobre el hospedero o parásito. Poulin (1995) sugiere al menos cuatro criterios para que modificaciones fenotípicas en hospederos sean consideradas como adaptaciones verdaderas.

(1) *Complejidad*: rasgos simples pueden ser adaptaciones, pero pueden también aparecer por azar como productos secundarios de otros cambios selectivos. Sin embargo, es poco probable que rasgos complejos sean el producto de accidentes del azar.

(2) *Propósito de diseño*: algunos caracteres adaptativos están muy bien ajustados a su función y su ambiente para aparecer por azar, y reflejan la fuerza de la selección natural.

(3) *Convergencia*: las adaptaciones pueden ser reconocidas a una escala macroevolutiva. Si diferentes linajes de parásitos con ciclos de vida similares han

evolucionado independientemente la habilidad para causar alteraciones idénticas en el fenotipo de hospederos, entonces seguramente esta habilidad es adaptativa.

(4) *Efectos sobre la adecuación biológica*: un carácter es adaptativo si se demuestra que conlleva un aumento de la adecuación biológica del portador.

Desde una perspectiva teórica, es esperable que exista variabilidad intra-poblacional de los parásitos en su habilidad para manipular la conducta de hospederos, especialmente si la manipulación conlleva costos asociados. Para que la capacidad de modificar el fenotipo de los hospederos sea adaptativa, los beneficios en adecuación biológica deben sobrepasar los costos incurridos. No obstante algunos parásitos pueden producir cambios en la conducta de hospederos sin incurrir en ningún gasto, simplemente por estar en el órgano apropiado por azar (Poulin 1998a). La mayoría de las alteraciones conductuales descritas en hospederos parecen ser el resultado de interferencia de neuroquímicos del hospedero mediante la secreción y liberación de hormonas y otros neuroquímicos por parte del parásito (Helluy & Holmes 1990; Hurd 1990; Thompson & Kavaliers 1994).

1.1 Interacciones entre parásitos y hospederos intermediarios

Las interacciones entre parásitos y hospederos intermediarios han sido extensamente documentadas en la literatura (Wilson & Edwards 1986; Curtis 1987; LoBue & Bell 1993; Bakker *et al.* 1997). Se ha sugerido que modificaciones en la morfología y conducta de hospederos intermediarios producto de la interacción con el parásito podrían tener un efecto importante en la probabilidad de transmisión del

parásito hacia un hospedero definitivo (Moore 1993). De hecho, se han descrito casos en que cambios en la morfología, tales como aumento en la conspicuidad (e.g., Bakker *et al.* 1997), y conducta (e.g., Lim & Green 1991; Levri & Lively 1996; Bakker *et al.* 1997; Thomas & Poulin 1998; Levri 1999; McCurdy *et al.* 1999; Berdoy *et al.* 2000) de hospederos intermediarios parasitados los tornan más susceptibles a ser localizados y consumidos por hospederos finales (Poulin 1998a). Adicionalmente, hospederos intermediarios infectados tienden a seleccionar sitios con características que facilitarían la dispersión del parásito aumentando la probabilidad de encuentro entre este último y su hospedero definitivo (e.g., Curtis 1987; Lowenberger & Rau 1994; Maitland 1994). La complejidad de algunos cambios conductuales inducidos por parásitos sugieren que ellos beneficiarían al parásito o al hospedero, y por lo tanto podrían ser consideradas adaptaciones legítimas para uno u otro interactuante. Por ejemplo, hongos que parasitan insectos provocan que sus hospederos mueran en la posición óptima para la dispersión de sus esporas (Maitland 1994). Algunos tremátodos digeneos hacen que sus hospederos intermediarios, gastrópodos, cambien de microhábitat y se muden al sitio ideal para la liberación de las cercarias (Curtis 1987; Lowenberger & Rau 1994). Otro caso es el documentado para el tremátodo digeneo *Dicrocoelium dendriticum*, transmitido desde una hormiga hacia una oveja vía ingestión. El parásito induce a la hormiga a migrar hacia la punta del pasto, aquel microhábitat forrajeado por la oveja, lo cual aumenta la probabilidad de transmisión a su hospedero definitivo (Poulin 1995). En este mismo grupo, un último caso ampliamente documentado es del tremátodo digeneo *Leucochloridium* que causa que los tentáculos de gastrópodos, sus hospederos intermediarios, cambien de tamaño, forma y color, y que palpiten en respuesta a la luz.

Estos cambios capturarían la atención de los hospederos definitivos, aves, quienes aumentan la tasa de depredación sobre tales ejemplares (Poulin 1995). Finalmente, nemátodos mermítidos inducen a sus insectos hospederos a ocupar microhábitats apropiados para la emergencia de estados adultos del parásito (Vance 1996). Alteraciones de la conducta del hospedero que siguen a la infección parasitaria es lo que se esperaría observar si el hospedero actuara de una manera que beneficie al parásito (Curtis 1987; Lowenberger & Rau 1994; Maitland 1994; Poulin 1995; Vance 1996), siempre y cuando estas alteraciones sean adaptaciones más que meras consecuencias patológicas colaterales. Sin embargo, sólo una pequeña fracción de los estudios de cambios conductuales de hospederos inducidos por parásitos han evaluado su efecto sobre la adecuación biológica de los participantes (Poulin 1998a). La mayoría de los estudios se han centrado en la detección de modificaciones fenotípicas en hospederos intermediarios y su consecuente aumento en la probabilidad de ser depredados por hospederos definitivos. Menos conocida es la situación en que la transmisión del parásito no depende de una interacción trófica entre hospederos intermediario-definitivo, sino de un contacto efectivo no depredatorio entre ambos participantes.

1.2 Modificaciones fenotípicas en insectos

Los insectos forman el grupo más diverso de organismos vivos y han sido reiteradamente colonizados por parásitos a lo largo de su historia evolutiva (Molyneux 1993). Diversos taxa de parásitos inducen variados cambios en sus insectos hospederos, tales como alteraciones en la selección de hábitat (e.g., Maitland 1994), en los niveles de

actividad (e.g., Moore *et al.* 1994), en los hábitos de forrajeo (e.g., Jenni *et al.* 1980; Roberts 1981; Beach *et al.* 1985; Rossignol *et al.* 1986; Koella *et al.* 1998; Anderson *et al.* 1999), en la agregación espacial (e.g., Wilson & Edwards 1986) y en la reproducción (e.g., Carmichael *et al.* 1993; Simmons 1993; Adamo 1999). En las últimas décadas, se ha dado particular énfasis a aquellos insectos que actúan como vectores (u hospederos intermediarios) de parásitos de importancia médica, tales como mosquitos (*Aedes* y *Anopheles*) y moscas tsetse (*Glossina*). Por ejemplo, se ha demostrado que protozoos del género *Plasmodium,* causantes de la malaria, modifican la persistencia de alimentación de sus vectores hembras pertenecientes al género *Anopheles* (Diptera: Anophelinae) (Rossignol *et al.* 1986; Wekesa *et al.* 1992; Koella *et al.* 1998; Anderson *et al.* 1999). Las especies del género *Plasmodium* son transmitidas mediante la saliva del mosquito al momento de picar a un hospedero definitivo. Experimentos efectuados por Koella *et al.* (1998) indican que tanto la frecuencia de picada como la duración de dicho evento (i.e., volumen de sangre obtenido) se modifica debido a la migración de *Plasmodium* hacia el aparato succionador del vector. Anderson *et al.* (1999) encontraron que la modificación en la conducta de alimentación de mosquitos depende del estadío de desarrollo del parásito, ocurriendo principalmente cuando el protozoo se encuentra en su estado transmisible (esporozoito). Por otro lado, Wekesa *et al.* (1992) documentaron que la conducta de alimentación de mosquitos hembras infectadas no dependía de los niveles de infección con esporozitos de *Plasmodium* spp. ni de la edad o estado reproductivo de las hembras. Estudios realizados en otras dos especies de moscas picadoras, *Phlebotomus* spp. (Diptera: Psychodidae) y *Aedes* spp. (Diptera: Culicinae), han demostrado modificaciones en las conductas de alimentación de individuos infectados

con los parásitos *Leishmania* spp. (Protozoa) y *Plagiorchis* spp. (Trematoda: Plagiorchiidae), respectivamente (Beach *et al.* 1985; Webber *et al.* 1987).

Alteraciones de la conducta de muchos insectos succionadores de sangre debido a infecciones con tripanosomátidos (Protozoa), aunque no tan conspicuas ni letales como aquellas inducidas por helmintos en sus hospederos intermediarios, parecen ser muy eficientes (Schaub 1992). Al respecto, existen dos mecanismos por los cuales los tripanosomátidos pueden aumentar el número de ataques de los insectos vectores sobre los dadores de sangre. (1) Los tripanosomátidos y el insecto vector compiten por metabolitos presentes en la sangre ingerida. La evidencia experimental indica que aquellos vectores que mueren por inanición y están infectados con *T. cruzi* presentan más remanentes de hemoglobina en el intestino que insectos no infectados, indicando que el deceso puede ser causado por un agotamiento de nutrientes traza, por los cuales el flagelado y el insecto estarían compitiendo (Schaub 1992; Kollien & Schaub 2000). (2) Los tripanosomas interfieren con el proceso de ingestión del vector. Estos efectos sobre insectos succionadores de sangre están conectados con perturbaciones del tracto digestivo, especialmente el intestino anterior y medio. Frecuentemente, vectores infectados succionan volúmenes de sangre muy pequeños, y por lo tanto necesitan alimentarse más veces y atacar a nuevos hospederos, aumentando así la probabilidad de transmisión del parásito (Molyneux & Jefferies 1986; Schaub 1992). Un caso bien estudiado es el de la mosca tsetse (Diptera: Glossinidae), insecto vector que transmite protozoos africanos del género *Trypanosoma*, parásito que invade el sistema nervioso causando la enfermedad del sueño en humanos. Los resultados presentados por Jenni *et*

al. (1980) indican que moscas tsetse (*Glossina* sp.) infectadas con *Trypanosoma* presentan diferencias en su conducta de alimentación, realizando un mayor número de intentos de picada y alimentándose más vorazmente, debido a una función deteriorada de los mecanorreceptores ubicados en el labrum.

Los resultados de los estudios realizados en insectos succionadores de sangre indican que los parásitos podrían modificar la conducta de hospederos intermediarios, incrementando así su probabilidad de transmisión hacia hospederos definitivos (i.e., la evidencia en el caso de insectos vectores apoya la hipótesis de manipulación). Notorio en tales estudios, sin embargo, es la ausencia de estimaciones del valor adaptativo de la modificación conductual.

1.3 Modificaciones fenotípicas y el sistema en estudio

En sistemas hospedero-parásito con participación de hospederos intermediarios y definitivos, la evidencia empírica indica que la modificación fenotípica de hospederos intermediarios puede favorecer la transmisión del parásito hacia hospederos depredadores definitivos mediante un incremento en la vulnerabilidad a la depredación. En este tipo de sistemas, la transmisión parasitaria ocurre en estrecha relación a una interacción depredador-presa. Sin embargo, los parásitos pueden aumentar su transmisión no sólo incrementando la vulnerabilidad del hospedero intermediario a la depredación, sino también mediante un aumento en la tasa de contactos efectivos no depredatorios entre el hospedero intermediario (vector) y el hospedero definitivo. La

transmisión parasitaria mediada por vectores puede ocurrir mediante la saliva del vector al momento de picar a su hospedero o mediante la deyección del vector sobre un hospedero picado. Al respecto, existe evidencia que apoya la idea que cambios conductuales en el proceso de alimentación inducidos por parásitos resultarían adaptativos para la transmisión parasitaria. Sin embargo, todos los estudios se han efectuado en sistemas con parásitos de transmisión salival, desconociéndose si los cambios conductuales son igualmente adaptativos en parásitos con transmisión mediante deyección.

Un estudio realizado por Añez & East (1984) examinó el efecto del protozoo *Trypanosoma rangeli* en la conducta de alimentación de *Rhodnius prolixus* (Hemiptera: Reduviidae). En este estudio los autores mostraron modificación en la conducta de alimentación del vector, específicamente un aumento en la tasa de picada de insectos infectados con *T. rangeli*. Al igual que otros vectores de parásitos de importancia médica, *T. rangeli* se transmite mediante la saliva del insecto al momento de picar. Por lo tanto, no existe evidencia empírica del efecto que puede tener un parásito que se transmite por deyección sobre la conducta de su vector.

Para sistemas tripanosomátido-triatomino existe solamente un estudio que indica que parásitos *Trypanosoma cruzi* podrían estar modificando el tiempo de desarrollo en ninfas de *Triatoma infestans* (Reis dos Santos & Lacombe 1985). Específicamente, insectos infectados demorarían más tiempo en alcanzar la madurez al compararlos con individuos sin la infección. Sin embargo, estudios posteriores realizados por Schaub (1992) no mostraron diferencias entre grupos infectados y no infectados, atribuyendo las diferencias observadas en el estudio previo a problemas de diseño experimental,

particularmente debido a crianza en aislamiento. De acuerdo a los resultados obtenidos por Schaub (1992), la mortalidad de estos insectos no sería afectada por la presencia del parásito protozoo. *Trypanosoma cruzi* estaría actuando como un agente de estrés sinérgico, que conduce a efectos adversos sólo si un segundo agente de estrés está presente. Bajo condiciones óptimas de alimentación la pérdida de metabolitos provocada por el parásito se compensaría con un aumento en el número de eventos de alimentación y/o del volumen de sangre ingerida (Juarez 1970).

Pocos estudios en sistemas tripanosomátido-vector han evaluado la sobrevivencia y la tasa reproductiva de insectos adultos. Un trabajo realizado por Schaub (1994) indicó que los tripanosomátidos no incrementan el tiempo de vida de insectos hospederos mediante castración o reducción de la reproducción como en otros sistemas de parásitos-hospederos intermediarios. Los estudios con *T. cruzi* han mostrado resultados contradictorios al evaluar si insectos adultos son afectados por la infección. Sin embargo, en el sistema *Trypanosoma cruzi-Triatoma infestans* ocurre una leve reducción en la tasa de postura de huevos durante las dos primeras semanas del período reproductivo y un decaimiento en la tasa de eclosión (Schaub 1994). Desafortunadamente, las comparaciones entre estudios son difíciles de realizar debido a que las condiciones experimentales, como por ejemplo frecuencia de alimentación, condiciones abióticas, niveles de hacinamiento, no son equivalentes. Un problema adicional es que la tasa reproductiva de estos insectos disminuye con la edad (Schaub 1992).

1.3.1 Biología del vector y parásito

Mepraia spinolai Porter, 1934 (Sinonimia: *Triatoma spinolai*), es un insecto perteneciente a la Subfamilia Triatominae (Hemiptera: Reduviidae). Las especies pertenecientes a esta subfamilia se caracterizan por (i) presentar hematofagia obligada, (ii) poseer un ciclo de vida hemimetábolo y (iii) requerir la sangre de un vertebrado para alcanzar el estado adulto. Su cuerpo es alargado, y aplastado dorsoventralmente en ayuno. El tamaño va desde 5 mm (instar I) hasta 4.5 cm (adulto). La coloración del cuerpo es café-plomizo (estadíos I al V) o negro con bandas de color anaranjado sobre el conexivo en el estado adulto (Lent & Wygodzinsky 1979).

Mepraia spinolai es una especie silvestre endémica de Chile (Schenone *et al.* 1980; Fotografías 1 & 2), que presenta un marcado polimorfismo, con hembras micrópteras y machos micrópteros, braquípteros o macrópteros (Canals *et al.* 1998). Se distribuye desde la zona norte (Paralelo 18) hasta los alrededores de Santiago (Paralelo 34) (Neghme 1982). Esta especie ha sido descrita en sectores abrigados y soleados, desde la cordillera (3000 msnm) hasta sectores costeros (Gajardo-Tobar 1960; Schenone *et al.* 1980). El hábitat de *M. spinolai* está constituido por zonas pedregosas, grietas en rocas o tierra, madrigueras de animales y güaneras de aves (Fotografía 3). En muy pocas ocasiones ha sido encontrado en viviendas humanas o en zonas periurbanas (Schenone *et al.* 1980). Este triatomino se alimenta principalmente de sangre de mamíferos y aves a cualquier hora del día, lo cual lo diferencia de otras especies de hábitos estrictamente nocturnos (Gajardo-Tobar 1960; Canals *et al.* 1997).

Los triatominos han sido motivo de estudio debido a que son participantes activos de la enfermedad de Chagas. Se han descrito 118 especies en el mundo, de las cuales 105 se encuentran en América, único continente donde la subfamilia ejerce su papel como vector (Schofield 1994; Galvao *et al.* 2003). Aproximadamente 53 de estas especies son portadoras de *Trypanosoma cruzi*, siendo la mayoría responsables de la mantención del ciclo silvestre de la enfermedad infecciosa (Zeledón 1974). Específicamente, para *M. spinolai* se ha descrito una prevalencia de infección con *T. cruzi* de 11.4-26.0% (Apt & Reyes 1990; Ordenes *et al.* 1996).

Trypanosoma cruzi, es un protozoo mastigóforo perteneciente a la familia Trypanosomatidae, en cuyo ciclo biológico intervienen insectos vectores y mamíferos (Atias & Apt 1991; Fotografías 4 & 5). Se transmite principalmente a través de vectores de la subfamilia Triatominae (80%) (Schofield 1994). Otras formas de transmisión en humanos son la vía transplacentaria, transfusión sanguínea y accidentes de laboratorio (Apt & Reyes 1986, 1990; Schenone & Rojas 1989; Schofield 1994). El parásito puede ser encontrado tanto en deyecciones de insectos infectados como en la sangre de hospederos vertebrados (Schenone & Rojas 1989).

En sus diversos hospederos y en medios de cultivo, *T. cruzi* presenta tres aspectos morfológicos fundamentales. (i) El tripomastigoto se encuentra en la sangre de mamíferos y en el intestino posterior de los triatominos. No se multiplica, pero constituye la forma infectante para los mamíferos y los triatominos. En los mamíferos, es el diseminador de la infección vía sanguínea. (ii) El epimastigoto es la forma de multiplicación del parásito en el intestino del triatomino. (iii) El amastigoto es la forma de multiplicación del parásito y lo lleva a cabo en el interior de las células del mamífero

(Atias & Apt 1991). Estudios más recientes de Kollien & Schaub (2000) describen 18 formas diferentes al considerar los estados intermedios más importantes de este protozoo.

El ciclo de transmisión de *T. cruzi* comienza cuando una vinchuca se infecta al ingerir sangre de un mamífero infectado con tripomastigotos. En el lúmen del intestino medio del insecto, los parásitos se multiplican muy activamente como epimastigotos por fisión binaria y, al cabo de 15 a 30 días, se desarrollan los tripomastigotos metacíclicos en el intestino posterior del triatomino. Cuando el insecto infectado pica al mamífero, emite deyecciones con tripomastigotos, los que atraviesan la piel por el sitio de la picadura o por las mucosas. En el mamífero, los tripomastigotos metacíclicos se introducen en las células del tejido celular laxo, vecino al sitio de penetración, y adquieren la forma de amastigotos. Los amastigotos se multiplican por fisión binaria, repletan la célula que termina por romperse, e ingresan los parásitos a la circulación bajo el aspecto de tripomastigotos, diseminándose por todo el organismo. El ciclo biológico se completa cuando los tripomastigotos son ingeridos por triatominos hematófagos (Atias & Apt 1991; Cox 1993).

1.3.2 Potencial de modificación fenotípica en el sistema en estudio

El sistema de estudio elegido está constituido por la vinchuca silvestre, *Mepraia spinolai* (Hemiptera: Reduviidae), y el parásito causante de la enfermedad de Chagas, *Trypanosoma cruzi* (Protozoa). Este sistema endémico permite abordar diversas

problemáticas de la interacción parásito-vector poco estudiadas en el caso de insectos triatominos.

En general, los estudios en triatominos que examinan los fenotipos de vectores infectados han incluido solamente uno de sus varios estadíos de desarrollo (e.g., Añez & East 1984). Sin embargo, existen varias razones para indicar que no todos los estadíos ontogenéticos del vector responden de igual manera al impacto de un parásito. Primero, la expresión de un cambio fenotípico en un hospedero intermediario (o vector) debiera depender del momento en que ocurre la infección, es decir, el tiempo que ha transcurrido desde que el parásito entró en contacto con el vector (tiempo acumulado de contacto). Segundo, ciertos estadíos de desarrollo pueden ser más sensibles al impacto del parásito, dependiendo de la asignación de energía a otras funciones relacionadas con la adecuación biológica. Por lo tanto, al no incorporar la variable ontogenética en estudios parásito-vector se podrían enmascarar aspectos importantes de la interacción. El primer propósito de esta tesis es examinar si el parásito *T. cruzi* modifica el tiempo de desarrollo de la vinchuca silvestre *Mepraia spinolai*, considerando el tiempo de muda acumulado y estadío-por-estadío. Adicionalmente, evalúo si el impacto de *T. cruzi* depende del sexo del vector. Específicamente, abordo las siguientes preguntas: (1) ¿Es el tiempo de desarrollo del vector *M. spinolai* afectado por el parásito *T. cruzi*? (2) ¿Son los distintos estadíos ninfales de *M. spinolai* afectados de la misma manera por *T. cruzi*? (3) ¿Son los fenotipos de vectores machos y hembras igualmente sensibles al parásito?

Durante el período de transmisión de los parásitos, las presiones selectivas debieran favorecer a aquellos parásitos que pueden manipular a sus vectores y así aumentar la probabilidad de transmisión (Hurd 2003). Debido a que *T. cruzi* se transmite

principalmente por autoinoculación realizada por el mismo hospedero definitivo, es importante evaluar la ocurrencia de sincronización entre la picada y la deyección por parte del insecto. Canals *et al.* (1999) estudiaron la conducta de alimentación de estadíos ninfales V en *M. spinolai*, y encontraron que solamente un 3.7% de los individuos deyectaban sobre la presa después de picar. Sin embargo, en dicho estudio no se indica el estatus de infección de *M. spinolai* con *T. cruzi*. El segundo propósito de esta tesis es estudiar los componentes de la conducta de *M. spinolai* que inciden en la transmisión del parásito y si la infección modifica alguno de ellos. Se examinan las conductas asociadas a la alimentación que están directa o indirectamente relacionadas al sistema de transmisión del protozoo.

Muchos parásitos de insectos reducen la salida reproductiva de sus vectores (Hurd 1998; Webb & Hurd 1999). La destrucción mecánica de las gónadas no es frecuente; usualmente la reducción en la producción de huevos se debe a cambios morfológicos, fisiológicos o conductuales del fenotipo del hospedero (Webb & Hurd 1999). El tercer propósito de esta tesis es evaluar el impacto del parasitismo en el desempeño reproductivo del vector *M. spinolai*. Se abordan las siguientes preguntas: (1) ¿Es el tamaño de los vectores adultos modificado por el parásito? (2) ¿Reduce el parásito el desempeño reproductivo de su insecto vector en términos de tasa de producción de huevos, porcentaje de eclosión, número/peso de huevos? (3) ¿Existe un compromiso entre cantidad y calidad de huevos y ninfas primer estadío producidas por los insectos? (4) ¿Son los vectores machos y hembras afectados de la misma manera por la infección?

1.4 Objetivos generales e hipótesis

1) Evaluar el efecto del protozoo parásito *Trypanosoma cruzi* sobre el tiempo de desarrollo de su insecto vector *Mepraia spinolai*. Con respecto a este objetivo se someterán a prueba las siguientes hipótesis:

1.1 Si la expresión de un cambio fenotípico en un vector es un efecto aditivo que depende del tiempo acumulado de contacto vector-parásito, a mayor tiempo transcurrido desde que el parásito contacta al vector, mayor debiera ser la probabilidad de observar una modificación en el fenotipo de este último (Capítulo 1).

1.2 Si los parásitos no modifican de igual manera el fenotipo de vectores machos y hembras, la modificación fenotípica debiera ser mayor en aquel sexo que presente mayores requerimientos energéticos (Capítulo 1).

1.3 Si la modificación fenotípica inducida por el parásito posee valor adaptativo para el vector, tal cambio debiera reducir el impacto en adecuación biológica atribuible al parasitismo (Capítulo 1).

2) Evaluar el efecto del parásito protozoo *Trypanosoma cruzi* sobre la conducta de alimentación de su insecto vector *Mepraia spinolai*. Con respecto a este objetivo se someterá a prueba la siguiente hipótesis:

2.1 Si los parásitos con transmisión por deyección aumentan su adecuación biológica modificando la conducta de alimentación de sus vectores (Hipótesis de manipulación), se debieran observar modificaciones en el proceso de alimentación del vector coincidentes con un aumento de la probabilidad de transmisión del parásito (Capítulo 2).

3) Evaluar el efecto del parásito protozoo *Trypanosoma cruzi* sobre la fecundidad de su insecto vector *Mepraia spinolai*. Con respecto a este objetivo se someterán a prueba las siguientes hipótesis:

3.1 Si el parásito disminuye la fecundidad de insectos infectados, se debiera esperar la menor producción, tamaño y viabilidad de huevos cuando uno de los parentales está infectado (Capítulo 3).

3.2 Si el parásito disminuye la fecundidad de insectos infectados dependiendo de su sexo, se debiera esperar un mayor efecto en el sexo con la mayor carga reproductiva, en este caso la hembra (Capítulo 3).

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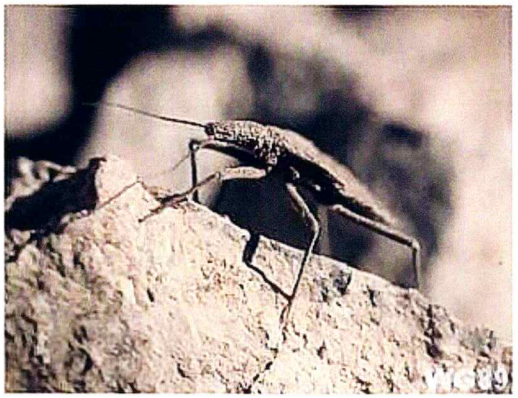
1.6 Fotografías

1. Ninfa de quinto instar de *Mepraia spinolai*
2. Hembra adulta de *Mepraia spinolai* (cortesía Mariana Acuña)
3. Hábitat de *Mepraia spinolai*, Reserva Nacional Las Chinchillas, Aucó, IV Región
4. Formas en el ciclo de vida de *Trypanosoma cruzi*: (a) promastigoto, (b) epimastigoto, (c) tripomastigoto, y (d) amastigoto (Cox 1993)
5. Microfotografía electrónica de *Trypanosoma cruzi* (Cox 1993)



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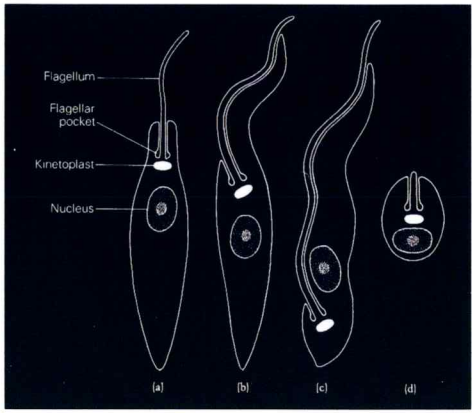

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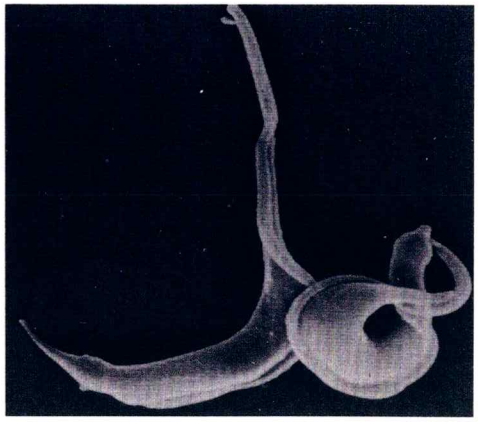

5

CAPÍTULO 1

2. Parasite-induced changes in the developmental time of a trypanosome-infected kissingbug

SUMMARY

The extent to which parasites manipulate the host phenotype to increase the probability of transmission, or the host phenotype change represents an incidental side effect of parasite infection is a contentious issue. In this paper I examine whether the protozoan parasite *Trypanosoma cruzi* modifies the developmental time of the kissingbug *Mepraia spinolai* (Hemiptera; Reduviidae). Results indicate that *T. cruzi*-infected bugs showed a slower moulting time than uninfected individuals. The impact of the parasite was age-dependent, as the last three moults were the most affected stages. In addition, *T. cruzi* decreased significantly the weight of males and females in the three last stages. When insect sex was taken into account, female kissingbugs took longer time than males to develop into the adult stage, which implies that the impact of *T. cruzi* is sex-dependent. These results indicate that *T. cruzi* modifies the phenotype of *M. spinolai*, and provide the first evidence for age and sex-dependent parasite-induced phenotype modification.

1. INTRODUCTION

Host-parasite interactions are often described as a resource-consumer relationship, where parasites maximize fitness, either by increasing the probability of completing its life cycle, or by increasing offspring production as a consequence of host infection. The way parasites exploit their host populations, however, can have different consequences for host fitness. On the one hand, parasites can reduce directly host fitness through increases in mortality and castration (Minchella 1985; Ballabeni 1995; Poulin 1998). On the other hand, subtle and more indirect parasite impacts such as host phenotype modifications, can lead to decreased host survivorship or reproduction after the new host phenotype is expressed. Even though these parasite-induced phenotype modifications have long attracted the attention of parasitologists, only recently the ecological and evolutionary consequences of these epigenetic modifications have been examined. Many studies have described that parasites can modify a wide range of physiological, behavioural and morphological host traits (Holmes & Bethel 1972; Moore & Gotelli 1990; Hechtel *et al.* 1993; Mouritsen & Jensen 1994; Ballabeni 1995; Moore 1995; Vance 1996; Poulin & Thomas 1999). However, whether these host alterations represent simple pathological side-effects of parasite infection, consequences of parasite manipulation to increase transmission to another host, or host adaptations to minimize the costs of being parasitized is still a source of controversy (Poulin 2000; Wilson 2000). Recent studies have warned that unless quantitative assessments reveal a significant overall effect of the host phenotype change on parasite fitness, the host change should not *a priori* be considered as parasite adaptations (Moore & Gotelli 1990; Poulin 2000;

Tompkins *et al.* 2004). In the absence of such evidence, the more parsimonious null hypothesis that altered host phenotype is simply an incidental side effect of infection cannot be rejected.

In this study, I focus in a trypanosomatid-insect relationship endemic to Chilean arid zones, that consists on the protozoan parasite *Trypanosoma cruzi* and its reduviid vector *Mepraia spinolai* (Lent *et al.* 1994). Several studies on Triatomine-trypanosomatid interactions have investigated whether parasites produce behavioural alterations, disturbances of organ systems, pre-adult development delay, reduction in lifespan and reproductive rate, and synergistic effects between the parasite itself and other stressors (e.g., Molyneux *et al.* 1978; Añez & East 1984; Reis dos Santos & Lacombe 1985; Schaub 1989; Giojalas *et al.* 1990). Even though results indicate that trypanosomatids have in general few pathological effects on insect vectors, most studies assume that insect developmental stage and sex do not influence the impact of *Trypanosoma* on the host phenotype. However, hosts of different ages and sexes may represent different resource environments for parasites because of the age-dependent energy budget and energy allocation pattern at the time of infection (Gerard & Theron 1997). For example, the weakening of insects by ageing or adverse weather conditions may increase the pathogenicity of the flagellates (Schaub 1994). Likewise, some parasites induce juvenilization and sexual reversal in males but not females (Tomalak *et al.* 1984; Hurd 1990), which suggests that overall conclusions on the importance of trypanosomatids in modifying the insect phenotype may be contingent to the host sex that is affected. In this paper, I evaluate the importance of *T. cruzi* in modifying the developmental time of *M. spinolai* on a cumulative and per instar basis. In addition, I

examine whether the phenotypic impact of *T. cruzi* is contingent to the host sex that is involved in the interaction. More specifically, I will address the following questions: (1) Does *T. cruzi* affect the developmental time of its vector *M. spinolai*? (2) Does *T. cruzi* modify the host phenotype evenly across host ontogeny? (3) Are male and female host phenotypes evenly sensitive to *T. cruzi*?

2. MATERIAL AND METHODS

(a) *Study system*

The kissingbug *Mepraia spinolai* (*Triatoma spinolai*) is one of the two triatomine species responsible of *Trypanosoma cruzi* transmission in arid and semiarid Chile (Lent *et al.* 1994). This strictly hematophagous and diurnal species distributes between 18° and 34° S, and its main habitat includes stay grounds, bird nests, rock crevices, and caves although it has been also found in rustic and abandoned houses (Lent & Wygodzinsky 1979; Schofield *et al.* 1982; Canals *et al.* 1997). *Mepraia spinolai* requires blood of vertebrates, such as rodents, foxes, rabbits, marsupials and birds, 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 an adult. Often one full engorgement is sufficient for molting from one larval instar to the next (Kollien & Schaub 2000). This species is the only conspicuously polymorphic of the triatomine, presenting macropterous, brachypterous, micropterous males, and micropterous females (Lent & Wygodzinsky 1979).

The protozoan parasite *T. cruzi* is the causative agent of Chagas disease. This heteroxenous trypanosomatid possesses a life cycle that involves several morphologically different stages such as amastigotes, epimastigotes, promastigotes, trypomastigotes and spheromastigotes (Kollien & Schaub 2000). Infection of definitive hosts occurs by contamination of mucous membranes with bug feces or urine, which contain the infectious metacyclic trypomastigote stage of the flagellate (Kollien & Schaub 1997). Once inside the insect vector, the trypanosomatid multiplies and differentiates in the digestive tract.

(b) ***Experimental design***

One hundred crossings were carried out between adult *M. spinolai* males and females captured as fifth instar nymphs at the Reserva Nacional Las Chinchillas (31°30' S, 71°06' W, IV Region, Chile). Eggs were daily isolated from the crossings jars, cleaned and placed in sterile plastic containers. Once the first instar nymphs emerged, a random assignment to treatment (infected with *T. cruzi*) or control (uninfected) was performed. Previous reports indicate that maintenance in isolation or in overcrowded conditions, may represent stress factors for triatomines (Schaub 1994). I minimized such effects by housing nymphs at individual 3.2 cm x 3.6 cm compartments in an 18-compartment plastic box (11.4 cm x 20.5 cm). Olfactive and visual stimuli among neighbors were allowed by setting small holes on the walls of compartments. Each compartment was provided with sandy bottom and a folded piece of paper as a refuge

for bugs. All insects were reared in a climatic chamber at 26° C, 65-70% relative humidity, and 14:10 h L:D cycle.

A total of 549 treatment nymphs were infected at the first feed 3 - 4 weeks after eclosion using *T. cruzi* infected laboratory mice (C3H strain). The *T. cruzi* strain used was isolated in May 2002 from *M. spinolai* collected at the Reserva Nacional Las Chinchillas. Trypanosomes in feces and urine of field-captured insects were used to infect mice by intraperitoneal inoculation. Since 2002, the strain has been maintained by cyclical transmission across mouse generations. A total of 523 control nymphs fed on uninfected laboratory mice 3 - 4 weeks after eclosion. Only engorged first instar nymphs were used in this study. After each moulting, all nymphs were starved for 3 weeks and then fed on mice until the adult moult. The experimental feeding opportunities used in this study try to resemble natural situations with only one engorging chance after moulting. Nymphs were weighted before and after the feeding event in an analytic scale (precision 0.1 mg). Interstadial developmental time and mortality were recorded on a daily basis until adults emerged, and sex was determined according to sexually dimorphic characters (Lent & Wygodzinsky 1979). To ensure bug engorgement, a mouse was arrested in a mesh cage and one bug at a time was allowed to feed on it. All stages of control nymphs were fed with uninfected mice. To ensure that treatment nymphs were truly infected, I examined the presence of *T. cruzi* in fecal samples of fifth instar nymphs by light microscopy (NIKON Diaphot-FXA), compressing 5 µl of fresh fecal drops between a slide and a 18 mm x 18 mm cover-slip. The presence of motile parasites in 50 microscopic fields was recorded using an x400 magnification. All bugs

fed with infected mice showed evidence of *T. cruzi* in their feces. Mouse parasitaemia was checked on a weekly basis, and only infected mice with increasing parasitaemia were used for bug feeding (Feilij *et al.* 1983). In order to avoid any host effect on insect development (e.g., blood quality, odour stimuli), 2-month old mouse males from the same genetic line were used for feeding purposes.

(c) ***Statistical analyses***

I analyzed moulting time (the number of days elapsed between the last meal and the next instar emergence) and nymph weight (the weight of each nymph 3 weeks after moulting) with two-way repeated-measures ANOVAs, with status and sex as main factors. Cumulative moulting time (the time elapsed from first-instar nymph to adult) was analyzed with a two-way ANOVA, with status and sex as main factors. The dependent variables were log-transformed for normality and checked for homogeneity of variance by using the F_{max} test (Sokal & Rohlf 1995). Because differences in insect weight can be attributable not only to an effect of *T. cruzi*, but also to between-individual variation in the volume of ingested blood, I compared the cumulative ingested blood (the total amount of blood ingested by each individual along its life cycle) between groups using a two-way ANCOVA with fifth nymph weight as covariate, and status and sex as main effects.

3. RESULTS

Over the entire study, only 36.5% of the initial first-instar nymphs reached maturity (123 males and 268 females). Consequently, all the subsequent analyses were performed using this restricted, but complete dataset. To avoid any confounding effect attributable to male polymorphism, only micropterous males (n = 117) were considered in analyses. When infected and control groups were considered separately, 27.1% and 45.1% nymphs completed their life cycle, respectively (Chi-squared test with Yates' continuity correction, $X_1 = 36.9$, $p < 0.001$). Surviving infected bugs presented a lower male frequency than uninfected bugs (22.8% versus 35.2%, respectively, Chi-squared test with Yates' continuity correction, $X_1 = 6.02$, $p = 0.014$), indicating that groups differed in the resulting male to female ratio (infected group = 1:3.4, uninfected group = 1:1.8).

The mean moulting time for infected and uninfected individuals at each nymphal stage are summarized in Table 1. Infected males required 7 - 25% more time on the average to moult than uninfected ones, but the reversal situation was observed for fifth nymphs reaching maturity, where control nymphs required 9% more time than treatment individuals. The same trend was observed for females. Infected females required 2 - 24% more time on the average to moult than control bugs. The same result was observed for cumulative moulting time data. Infected males and females required 18 and 55 extra days on the average, respectively, to reach maturity than control individuals (table 1).

Results revealed a significant effect of infection on the moulting time. Infected insects required more time to moult through their ontogeny in comparison to uninfected

individuals (figure 1*a*,*b*). Similarly, there was a significant effect of sex on the developmental time of bugs. Males required less time to moult than females (table 1, figure 1*a*,*b*), and the greatest difference between sexes occurred when moulting from IV to V instar. The significance of time revealed that younger nymphs required less time to moult to the next developmental stage. The only significant interactions were status x time ($F_{4, 1300} = 2.65$, $p < 0.05$) and sex x time ($F_{4, 1300} = 9.13$, $p < 0.001$), suggesting that the significance of main effects are contingent to the instar stage that is considered (figure 1*a*,*b*). A second analysis revealed a significant effect of infection on the mean weight of 3 week starved nymphs. Uninfected insects were heavier than infected individuals (figure 1*c*,*d*). Fourth, fifth and adult instars showed the larger differences. There were significant effects of sex and time on the insect weight. Females were heavier than males, and older instars heavier than younger ones (Lent & Wygodzinsky 1979). In this analysis the only significant interactions were status x time ($F_{4, 1268} = 13.49$, $p < 0.001$) and sex x time ($F_{4, 1268} = 29.92$, $p < 0.001$) (figure 1*c*,*d*).

Analysis on cumulative moulting time revealed about the same results as above, with strong effects of status and sex on moulting time (Two-way ANOVA: status, $F_{1,325} = 22.124$, $p < 0.001$; sex, $F_{1,325} = 7.205$, $p < 0.01$; status and sex, $F_{1,325} = 4.570$, $p < 0.05$). However, unlike the previous analysis, there was a significant status x sex interaction suggesting that the effect of *T. cruzi* expresses on sexual differences in cumulative rather than in instar-dependent moulting time. While control males did no differ from control females, moulting time differed between sexes in infected *M. spinolai*, females requiring 42 extra days on the average to reach maturity (table 1). Finally, analysis on the cumulative ingested blood revealed a strong effect of sex ($F_{1, 316} = 84.84$, $p < 0.001$), but

neither status ($F_{1, 316} = 0.93$, $p = 0.34$) nor the sex x status interaction ($F_{1, 316} = 2.90$, $p = 0.09$), influenced the total amount of blood ingested by the insects. Therefore, even though female vectors ingested more blood than males, the parasite had no effect on blood intake in *M. spinolai* when standardized by insect weight (mean (mg) ± s.e., Females: treatment: 504.5 ± 10.7, control: 625.0 ± 12.3; Males: treatment: 382.9 ± 14.4, control: 439.1 ± 9.5).

4. DISCUSSION

Results from this study indicate that *T. cruzi*-infected kissingbugs are smaller and need more time to reach maturity than uninfected bugs. Even though the physiological mechanisms underlying this pattern are unknown at present, previous studies have suggested that trypanosomatids may compete for trace nutrients otherwise entirely available for triatomines (Kollien & Schaub 2000), and a critical level of metabolite concentration concerned with moulting is needed to initiate the hormonal induction of moulting (Schaub 1992). The impact *T. cruzi* has on the developmental time of their insects hosts seems to be a more widespread phenomenon than previously thought. For example, Reis dos Santos & Lacombe (1985) found that *T. cruzi* retards the larval developmental time in the pre-adult stages of infected *Triatoma infestans* (but see Schaub 1989). It is possible that *T. cruzi* acts as a subpathological stressor that translates in a small size and retarded developmental time of infected hosts when feeding opportunities are restricted (see also Schaub 1992).

One potential consequence of the retarded developmental time shown by infected kissingbugs is that trypanosomes increase spread toward definitive vertebrate hosts through increases in the number of feeding events made by kissingbugs to reach maturity. Several studies have shown that infected bloodsucking insects attack their hosts more often than uninfected ones, which would translate into higher disease transmission (Jenni *et al.* 1980; Añez & East 1984). Trypanosomatids and insect hosts seem to compete for metabolites in the ingested blood, making the insects to be hungry earlier (Schaub 1994). When the insect vectors die from starvation, more remnants of hemoglobin are present in the gut of *T. cruzi*-infected than uninfected insects, suggesting that the cause of death can be due to trace nutrient depletion (Kollien & Schaub 2000). However, when *T. cruzi* - infected insects are raised under optimum feeding conditions, the reduction in the amount of metabolites seem to be compensated by an increase in the number of feeding events and/or the ingested blood volume (Schaub 1992). Likewise, it has been suggested that established trypanosomes are capable of regulating their own population size and quality through programmed cell death, which may ameliorate resource competition with hosts, and hence extend the host lifespan to increase opportunities for transmission (Billingsley 1998). This situation has been observed in other trypanosomatids such as *Trypanosoma rangeli* and *Blastocrithidia triatomae* infecting species from the genus *Rhodnius*. For example, *T. rangeli* - infected *Rhodnius prolixus* needed 10-40% more time to reach the adult stage compared to uninfected insects. In this case, *T. cruzi*-infected bugs experience a 1.5-fold biting rate increase in comparison to uninfected insects (Botto-Mahan *et al.*, in prep.), which verifies partly the idea of increased parasite transmission.

The impact of the protozoan parasite on the phenotypic change of *M. spinolai* was age-related, and stronger in older rather than younger instars. Two non-mutually exclusive explanations may help to understand these results. On the one hand, because the body growth rate of parasitized insects tend to decline as infection proceeds (Hurd 1990), which is often accompanied by impaired food consumption and a concomitant alteration in gross conversion efficiency (Thompson 1983), it is possible that cumulative parasite loads acquired through successive feeding events along insect ontogeny translate into late rather than early phenotype change expression. On the other hand, it is possible that infected nymphs closer to maturity carry out an extra energy expenditure related to reproduction than younger infected nymphs. Theory predicts that when two or more life-history traits compete for resources, a trade-off arises because individuals cannot allocate resources to one trait without a concomitant reduction in allocation to a competing trait (Stearns 1989, 1992). Parasites are being recognized as an important factor influencing life-history evolution (Møller 1997). It is widely known that parasites have the potential to decrease reproductive output of hosts by competing for nutrients or forcing hosts to invest in immune function. When resource allocation to reproduction is still favored, allocation to somatic growth, self-maintenance, and immune function may be severely compromised. The extent to which *T. cruzi* impacts on the energy budget of *M. spinolai*, and influences resource allocation to body mass and moulting need to be assessed in future studies.

Analyses of cumulative moulting time revealed a strong sex x status interaction, indicating that the magnitude of the impact of *T. cruzi* on moulting time was sex-dependent, with females being more affected than males. In addition to the potential

fitness advantage for parasite transmission, if the parasite-induced phenotypic change has fitness consequences for *M. spinolai*, this strategy may represent an alternative way by which bugs tolerate the resource limitation imposed by *T. cruzi*. Hence, parasite-mediated selection for plastic life history strategies may allow hosts to compensate for some of the fitness losses attributable to parasitism. Because strategies to avoid or tolerate parasitism are often sex-specific as a consequence of sex differences in the resource allocation trade-off between parasite defense and other components of the phenotype (Tschirren *et al.* 2003), it is possible that reproductive allocation compromises development and body growth mainly in female rather than male individuals. The resolution of these trade-offs associated with tolerance and reproduction will be a result of the selection pressures imposed by parasites on the separate sexes. A better understanding of the host fitness costs and parasite benefits will help us to understand the adaptive significance of parasite-induced phenotypic change both from the host and parasite perspectives.

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Table 1. Summary of the mean moulting time (days ± 1 s.e.) of *T. cruzi*-infected and uninfected *Mepraia spinolai*. Parenthesis indicates sample size.

	Males		Females	
Stage	infected	uninfected	infected	uninfected
I→ II instar	20.32 ± 1.18 (34)	17.80 ± 0.27 (83)	20.36 ± 0.57 (115)	18.78 ± 0.29 (153)
II→ III instar	22.79 ± 1.07 (34)	21.12 ± 0.34 (82)	23.40 ± 0.59 (112)	22.97 ± 0.46 (148)
III→ IV instar	30.97 ± 1.55 (33)	25.85 ± 0.43 (81)	35. 51 ± 1.36 (112)	29.57 ± 1.07 (152)
IV→ V instar	62.15 ± 9.68 (26)	46.45 ± 3.27 (82)	88. 63 ± 5.39 (98)	67.51 ± 3.79 (150)
V → adult	87.24 ± 9.91 (33)	94.71 ± 6.89 (83)	84.66 ± 7.83 (111)	72.43 ± 5.74 (150)
I → adult	222.86 ± 12.17 (21)	204.98 ± 6.21 (79)	264.13 ± 6.93 (88)	208.90 ± 4.63 (141)

FIGURE CAPTION

Figure 1. Effects of *Trypanosoma cruzi* parasite on *Mepraia spinolai* vector. Mean (± 1 s.e.) moulting time of (*a*) female and (*b*) male vectors. Two-way repeated-measures ANOVA: status, $F_{1,325} = 72.568$, $p < 0.001$; sex, $F_{1,325} = 3.920$, $p < 0.05$; time, $F_{4,1300} = 252.772$, $p < 0.001$. Mean (± 1 s.e.) starved weight of (*c*) female and (*d*) male vectors. Two-way repeated-measures ANOVA: status, $F_{1,317} = 54.05$, $p < 0.001$; sex, $F_{1,317} = 28.43$, $p < 0.001$; time, $F_{4,1268} = 6524.34$, $p < 0.001$. Closed and open circles represent *T. cruzi*-infected and uninfected vectors, respectively.

mean moulting time (days)
mean starved weight (mg)
(a)
(b)
(c)
(d)
0
20
40
60
80
100
120
140
160
180
200
II
III
IV
V
A
instar

CAPÍTULO 2

3. Parasite-induced changes in the feeding behaviour of a *Trypanosoma cruzi*-infected triatomine: implications for parasite transmission

SUMMARY

Parasites have been hypothesised to manipulate the feeding behaviour of their invertebrate vectors to increase the probability of transmission to definitive hosts. Partial support for this hypothesis comes from protozoan species with salivary transmission. I present laboratory evidence that infection of the kissingbug *Mepraia spinolai* (Hemiptera; Reduviidae) with the dejection-transmitted protozoan *Trypanosoma cruzi* reduced the time needed for host vertebrate location, increased insect biting attempts, and decreased the blood intake from the vertebrate host. The time elapsed between blood intake and dejection was reduced in the presence of the parasite, suggesting that parasite-mediated changes in the feeding behaviour and dejection pattern of *M. spinolai* may promote the spread of trypanosomes toward definitive hosts.

1. INTRODUCTION

The way parasites induce host changes that increase the likelihood of parasite transmission has long attracted the attention of parasitologists and evolutionary ecologists (Poulin 1998; Moore 2002). Many studies have described that parasites can modify a wide range of physiological, behavioural, and morphological host traits (Holmes & Bethel 1972; Moore & Gotelli 1990; Hechtel *et al.* 1993; Mouritsen & Jensen 1994; Ballabeni 1995; Moore 1995; Vance 1996; Poulin & Thomas 1999). However, whether these host alterations represent simple pathological side-effects of parasite infection, consequences of parasite manipulation to increase transmission to another host, or host adaptations to minimize the costs of being parasitized is still a source of controversy (Poulin 2000; Wilson 2000).

In general, changes in host behaviour or appearance, are related to the mode of parasite transmission such as ingestion of the intermediate host/parasite, surface contact between succesive hosts, or inoculation of parasites by intermediate hosts (Hurd 1990). Examples of transmission by inoculation are hematophagous vectors, which exhibit altered feeding behaviour and increased blood parasite transmission (Molyneux & Jeffries 1986; Moore 1993; Hurd 2003). In vectors, the foraging process is central to parasite transmission and reported host modifications include the promotion of salivary pathology (Schaub 1992), an increase in probing behaviour (Killick-Kendrick *et al.* 1977; Añez & East 1984; Beach *et al.* 1985; Wekesa *et al.*1992), and a decrease in blood location ability (Moore 2002).

In trypanosome-insect associations, two mechanisms have been suggested to explain the increase in the number of attacks on hosts by bloodsucking insects. First, trypanosomatids and insect hosts compete for metabolites in the ingested blood, and resource depletion would lead to new feeding attempts (Schaub 1992). Partial support for this mechanism has been reported. For example, phlebotomine flies infected with bat trypanosomes seem to compete for metabolites essential for egg development (Williams 1976). Likewise, trypanosome-infected triatomines might compete for trace nutrients as evidenced by the higher amount of hemoglobin remnants in the gut of infected bugs in comparison to uninfected bugs (Kollien & Schaub 2000). Second, the trypanosomes interfere with the ingestion process by means of digestive tract disturbances, especially the foregut and the anterior midgut, promoting new feeding attempts (Schaub 1992). Evidence for this mechanism comes from *Leishmania*-sandfly associations, a system with salivary parasite transmission, where cases of blocked foreguts have been reported. The pharynx of sandflies can be blocked for its entire length with a plug of parasites implying that only small quantities of blood can be uptaken. Infected sandflies continue trying to obtain a bloodmeal at the same or different location increasing the chance of parasite transmission in comparison to infected sandflies that engorge successfully (Killick-Kendrick *et al.* 1977; Schaub 1992). Another feeding alteration produced by a salivarian-transmitted parasite was reported by Jenni *et al.* (1980), where *Trypanosoma congolense*-infected tsetse flies seemed to be more voracious and probed significantly more times than uninfected flies (but see Moloo 1983).

Few studies have examined the effect of trypanosomes on triatomine feeding behaviour (see review in Schaub 1992). Añez & East (1984) showed that the probing

behaviour of the triatomines *Rhodnius robustus* and *R. prolixus* was increased by infection with the salivarian-transmitted *Trypanosoma rangeli*. Moreover, infected insects fed for longer periods than uninfected individuals. In this study I focus on a trypanosomatid-insect system, the protozoan parasite *Trypanosoma cruzi* and its reduviid vector *Mepraia spinolai*, an endemic association from the arid zones of Chile (Lent *et al.* 1994). In this system, infection of definitive vertebrate hosts occurs by contamination of mucous membranes with bug feces or urine, which contain the infectious metacyclic trypomastigote stage of the flagellate (Kollien & Schaub 1997). Once inside the insect vector, the trypanosomatid multiplies and differentiates in the digestive tract. This endemic trypanosome-triatomine association allows us to examine the effect of the infection not only on the behaviour related to blood intake but also the dejection pattern of bugs. Because *T. cruzi* is a dejection-transmitted parasite, the most critical aspect of parasite transmission is the synchrony between blood ingestion and dejection.

In this paper, I assessed the effect of *Trypanosoma cruzi* on the feeding process of its vector *Mepraia spinolai*. More specifically, I explore the following questions: (1) does the parasite modify the time needed by the vector to detect and contact a potential definitive host? (2) Does the parasite modify the number of bites, feeding time, and biting rate of its triatomine vector? (3) Does the parasite synchronize blood intake and dejection by its vector? (4) Are male and female vectors evenly affected by the infection? If behavioural alterations are detected, a more general question can be addressed: (5) do behavioural modifications increase parasite transmission?

2. MATERIAL AND METHODS

(a) *Study system*

The kissingbug *Mepraia spinolai* (*Triatoma spinolai*) is one of the two triatomine species responsible of *Trypanosoma cruzi* transmission in arid and semiarid Chile (Lent *et al.* 1994). This strictly hematophagous and diurnal species distributes between 18° and 34° S and its main habitat includes stay grounds, bird nests, rock crevices, and caves although it has been also found in rustic and abandoned houses (Lent & Wygodzinsky 1979; Schofield *et al.* 1982; Canals *et al.* 1997). *Mepraia spinolai* requires blood of vertebrates, such as rodents, foxes, rabbits, marsupials and birds, 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 larval instar to the next (Kollien & Schaub 2000). This insect is the only conspicuously polymorphic species within triatomines, with macropterous, brachypterous, micropterous males, and micropterous females (Lent & Wygodzinsky 1979). The protozoan parasite *Trypanosoma cruzi* is the causative agent of Chagas disease. This heteroxenous trypanosomatid possesses a life cycle that involves several morphologically distinct stages that can be found in insect vectors and mammalian hosts (Kollien & Schaub 2000).

(b) *Infected and uninfected fifth instar nymphs*

Mepraia spinolai eggs were obtained from crossings between adult males and females captured as fifth instar nymphs at the Reserva Nacional Las Chinchillas (31°30'

S, 71°06' W, IV Region, Chile). Eggs were daily isolated from the crossings jars, cleaned and placed in sterile plastic containers. Once the first instar nymphs emerged, a random assignment to treatment (infected with *T. cruzi*) or control (uninfected) was performed. Each nymph was individually housed in a 3.2 cm x 3.6 cm clear plastic compartment of an 18-compartment box (11.4 cm x 20.5 cm). Each individual compartment was provided with sandy bottom and a folded piece of paper as refuge for the bug. All insects were reared in a climatic chamber at 26° C, 65-70% relative humidity, and 14:10 h L:D cycle.

Treatment nymphs were infected at the first feed 3 - 4 weeks after eclosion using *T. cruzi* infected laboratory mice (C_3H strain). The *T. cruzi* strain used was isolated in May 2002 from *Mepraia spinolai* collected at the Reserva Nacional Las Chinchillas. Trypanosomes in feces and urine of field-captured insects were used to infect mice by intraperitoneal inoculation. Since 2002, the strain has been maintained by cyclical transmission across mouse generations. Control nymphs fed on uninfected laboratory mice 3 - 4 weeks after eclosion. After each moult, infected and uninfected nymphs were starved for 3 weeks and then fed on infected and uninfected mice, respectively, until obtaining fifth instar nymphs. To ensure bug engorgement, mice were arrested in a mesh cage and one bug at a time was allowed to feed on it. To ensure that treatment nymphs were truly infected, I examined the presence of *T. cruzi* in fecal samples of fifth instar nymphs by light microscopy (NIKON Diaphot-FXA), compressing 5 µl of fresh fecal drops between a slide and a 18 mm x 18 mm cover-slip. The presence of motile parasites

in 50 microscopic fields was recorded using an x400 magnification. All bugs fed with infected mice showed evidence of *T. cruzi* in their feces.

(c) *Experimental design*

Experiments were carried out seven weeks after bugs moulted to fifth instar. Treatment and control insects were starved since their last bloodmeal as fourth instar. Experiments of 101 infected and 155 uninfected fifth instar nymphs were performed inside an experimental chamber provided with a video camera recorder (SONY DCR-TRV27). The chamber was artificially lighted and temperature and relative humidity controlled, 26° C and 70%, respectively. At the beginning of the experiment the nymph was kept under an upside down black tube placed on one randomly assigned corner of a square glass recipient (20.7 cm x 20.7 cm). After a 5 min-habituation, insects were released and allowed to feed on an uninfected anaesthetised mouse placed at the center of the recipient. Recording started with bug locomotion and ended with bug dejection. In order to avoid any host effect on insect behaviour (e.g., odour stimuli), only 2-month old male mice (*ca* 30 g) from the same genetic line were used for feeding purposes. Mice were used as host once a week. Nymph weight was recorded at the beginning and end of the feeding experiment.

Respect to host location, the following vector behavioural traits were measured: (i) vector activation (i.e., time elapsed between the beginning of the experiment and insect locomotion), (ii) orientation to the host (i.e., time elapsed between activation and antenna orientation), and (iii) contact (i.e., time elapsed between activation and host

contact). Respect to feeding behaviour, the following variables were measured: (i) rostrum ejection (i.e., time elapsed between activation and extension of rostrum), (ii) first bite (time elapsed between activation and first bite), (iii) total number of bites, (iv) feeding time (including all feeding bites), (v) biting rate (total number of bites/total feeding event), and (vi) blood intake (amount of blood ingested, measured as the difference between post-feeding nymph weight and pre-feeding nymph weight). The total feeding event corresponded to the time elapsed between the first bite and the end of blood ingestion. Respect to dejection pattern, the variable examined was the time of dejection, measured as the time elapsed between the end of blood ingestion and fecal drop deposition. The end of blood ingestion was considered as the moment of cessation of biting attempts after engorgement.

(d) ***Statistical analyses***

The variables related to host location were analyzed in a two-way MANCOVA, with status of infection and host sex as single factors and starvation period as covariate. To examine effects on each dependent variable, two-way ANCOVAs were performed with status and sex as single factors and starvation period as covariate. Starvation period was computed as the number of days elapsed between the last blood ingestion, i.e., engorgement as fourth instar nymphs, and the blood ingestion during the feeding experiment.

The variables related to feeding behaviour were analyzed in a two-way MANOVA, with status of infection and host sex as single factors. To examine the separate effects on each dependent variable, two-way ANOVAs were performed with status of infection

and host sex as single factors. For dejection pattern, the time of dejection was analyzed with a two-way ANCOVA, with status and sex as fixed effects and feeding time as covariate. All response variables were checked for homogeneity of variance by using the F_{max} test. To obtain normality, variables were log or square root-transformed (Sokal & Rohlf 1995).

3. RESULTS

A total of 101 and 155 experiments of *T. cruzi*-infected and uninfected *M. spinolai*, respectively, were carried out. Because insect behaviour may be contingent to the host sex that is examined, once fifth instar nymphs reached maturity, sex was established according to diagnostic characters (Lent & Wygodzinsky 1979; Frias *et al.* 1987). Treatment experiments included 60 females and 41 males, and control experiments included 86 females and 69 males. Initial weights of unfed infected and uninfected insects differed significantly between treatments (Mean (mg) ± 1 s.e.: infected: males = 32.65 ± 1.34, females = 41.14 ± 1.14; uninfected: males = 47.38 ± 1.48, females = 61.06 ± 1.65. Two-way ANOVA: Status: $F_{1,251} = 141.89$, $p < 0.001$; Sex: $F_{1,251} = 61.25$, $p < 0.001$). Overall, females were heavier than males, and infected bugs presented smaller weights than uninfected individuals.

(a) *Host location*

Results from a two-way MANCOVA on host location variables showed significant differences between infected and uninfected bugs (Wilks'lambda = 0.96, p = 0.03). However, no significant effect was detected between males and females (Wilks'

lambda = 0.99, $p = 0.52$). Two-way ANCOVAs revealed that infected bugs detected and orientated to mouse hosts almost twice faster than those uninfected, but activation and contact did not differ between experiment and control (tables 1 and 2). Sex had no significant effect on host location variables (table 2).

(b) ***Feeding behaviour***

Results from a two-way MANOVA showed significant effects of infection status and sex on the feeding behaviour variables (Status: Wilks' lambda = 0.80, $p < 0.001$; sex: Wilks' lambda = 0.66, $p < 0.001$). On the average, infected insects ejected their rostrum 43 sec faster than control insects, bitted 27% more times than uninfected insects, and had a biting rate 45% higher than controls (tables 1 and 3). When sex was considered, females needed 6 extra minutes to engorge than males (i.e., feeding time; tables 1 and 3). Likewise, the blood intake depended on the infection status and host sex (table 1 and 3). Infected insects ingested 29% less blood than uninfected individuals, and females ingested 52% more blood than males.

(c) ***Dejection pattern***

A two-way ANCOVA revealed significant effects of infection status and sex on dejection pattern (Status, $F_{1,243} = 7.24, p = 0.008$; sex, $F_{1,243} = 6.09, p = 0.014$; status and sex, $F_{1,243} = 0.595, p = 0.441$). Infected insects dejected 3 min faster on the average than those uninfected individuals. In terms of sex differences, males dejected 1 min faster on the average than females (table 1).

4. DISCUSSION

In this study I examined the effect of *Trypanosoma cruzi* on three consecutive components of the feeding process of its bloodsucking vector *Mepraia spinolai*. Results from several parasite-insect associations with salivary parasite transmission converge in documenting an altered foraging pattern (Moore 2002). The mechanisms responsible of such alterations range from physiological blockage and salivary gland pathology to sensory interference and food depletion (Schaub 1992). Unlike previous studies, however, this research has focused on a *T. cruzi*-transmission by fecal dejection that implies that other aspects of the foraging and feeding behaviour need to be examined to assess parasite effect.

Host location

A global analysis showed that even though infected and uninfected bugs differed on host location traits, no differences were detected between male and female insects. Separate analyses indicated that infected bugs detected and orientated to mouse hosts almost twice faster than those uninfected regardless of the sex considered, and excluding any starvation effect. Previous studies have reported that competition between trypanosomes and triatomine insects may result in hungry bugs more alert to potential host stimuli (Schaub 1992; Kollien & Schaub 2000). The 1.5-fold reduction in the body weight of *T. cruzi*-infected *M. spinolai* compared with control bugs provides partial support for this proximal explanation. Parasite-induced alterations in host location ability had not been previously reported for trypanosome-triatomine systems.

Feeding behaviour

A global analysis indicated significant effects of infection status and sex on feeding behaviour variables. *Trypanosoma cruzi*-infection and sex affected different aspects of the feeding behaviour. For example, rostrum ejection, number of bites, and biting rate depended only on infection status. Infected insects became ready to probe before than uninfected individuals. Similar to other parasite-vector systems (see review in Moore 2002), *T. cruzi*-infected insects increased the number of bites and the biting rate as compared to control insects. Because *T. cruzi* transmission occurs through dejection, no physiological blockage or salivary gland pathology can be invoked to explain this altered behaviour. Once inside the triatomine vector, *T. cruzi* colonizes the small intestine and rectum of the insect (Kollien & Shaub 2000), which turns unlikely that a migration of parasites occurs toward the foregut and anterior midgut at the time of bloodfeeding. Again, this altered behaviour can be a mere consequence of a higher level of starvation of infected bugs due to trace nutrient depletion, which translates into infected bugs eager to bite.

The time require to become engorged was higher for females than males, regardless of the infection status. This result is not surprising for two reasons. First, females are 1.3-fold larger on the average than males. If females and males ingest blood at the same rate, females would need more time to engorge because of size differences. Second, females of this triatomine species have a specialized connexivum with a large expansive membranous area that separates the ventral connexival plates from the urosternites (Lent & Wygodzinsky 1979). This large membranous area would allow females to ingest larger volumes of blood implying longer feeding periods.

The amount of blood uptaken was dependent of infection status and host sex. The finding that infected insects ingested less blood than uninfected could, in principle, be explained by the smaller weight shown by infected bugs. When the body weight effect was removed, this effect disappeared ($p = 0.89$), supporting the idea that alteration in this trait is not necessarily the result of a direct influence of *T. cruzi* on the feeding behaviour of its vector. On the contrary, an indirect side effect of parasite-mediated body weight reduction could be the most likely explanation.

Impaired feeding may have several consequences for hematophagous insect fitness. For example, infected insects may be malnourished, produce smaller egg clutches, and incur more risk from defensive hosts as they attempt to feed (Moore 2002). Reduction in mean egg weight has been observed for *T.cruzi*-infected *Mepraia spinolai*, which suggests that feeding alterations due to parasitic infection could translate into fitness consequences (Botto-Mahan *et al.*, in prep).

Dejection pattern

The time elapsed between the end of blood ingestion and fecal drop dejection was affected by infection status and host sex. In general, male insects dejected 1 min faster on the average than females. It is possible this effect reflects the fact that females have a larger anterior midgut than males to storage bloodmeals (Kollien & Schaub 2000).

The most remarkable finding of this study is that infected insects dejected 3 min faster on the average than uninfected controls. When insects deject, the metacyclic trypomastigotes are released and parasite transmission can occur. Apparently, *T. cruzi*

decreases the time between blood ingestion and dejection of *M. spinolai*. If this reduction in dejection time and the increase in the number of bites emitted by the vector are considered together, the probability of parasite transmission may increase substantially because more bites imply more potential wounds for parasite contamination. It is possible that behavioural changes result from competition between trypanosomatids and insect host by metabolites in the ingested blood, making the insect hungry earlier. On the other hand, because *T. cruzi* locates in the small intestine and rectum of its vector, the protozoan could accelerate the dejection process by releasing itself from the rectum in the presence of fresh bloodmeal, or by parasitic excretion of laxative compounds.

In summary, in this study I presented evidence of parasite-induced changes in the feeding process of *M. spinolai*. Most of these behavioural alterations are probably mere side effects of the infection, as a consequence of a resource curtailment experienced by the insect during its ontogeny. However, the modification in the dejection pattern can be the result of direct *T. cruzi* interference in the posterior intestinal region of the insect vector.

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Table 1. Descriptive statistics (mean ± s.e.) of variables related to host location, feeding behaviour and dejection pattern in *Mepraia spinolai*. Female sample size: uninfected = 86, infected = 60. Male sample size: uninfected = 69, infected = 40.

Variable	Uninfected	Infected
Activation (min)		
female	1.64 ± 0.22	1.38 ± 0.31
male	1.15 ± 0.18	1.25 ± 0.32
Orientation (min)		
female	0.43 ± 0.16	0.31 ± 0.09
male	0.96 ± 0.29	0.41 ± 0.21
Contact (min)		
female	1.96 ± 0.32	1.79 ± 0.28
male	3.52 ± 0.51	2.47 ± 0.64
Rostrum ejection (min)		
female	2.72 ± 0.36	2.19 ± 0.32
male	4.31 ± 0.52	2.99 ± 0.65
First bite (min)		
female	3.52 ± 0.41	3.17 ± 0.42
male	5.36 ± 0.57	3.57 ± 0.65
Feeding time (min)		
female	25.39 ± 1.53	22.54 ± 1.99
male	18.02 ± 1.08	18.54 ± 1.53
Number of bites		
female	5.33 ± 1.42	7.42 ± 1.48
male	4.32 ± 0.79	7.30 ± 2.04
Biting rate (number x min^{-1})		
female	0.16 ± 0.02	0.25 ± 0.03
male	0.18 ± 0.02	0.28 ± 0.04
Blood intake (mg)		
female	397.32 ± 12.18	286.17 ± 9.31
male	241.56 ± 5.57	206.22 ± 10.05
Time of dejection (min)		
female	11.47 ± 2.28	8.33 ± 1.14
male	9.99 ± 1.18	7.63 ± 1.01

Table 2. Summary of effects of *Trypanosoma cruzi*-infection on variables related to host location in *Mepraia spinolai*. All comparisons were performed by two-way ANCOVAs, with starving period as covariate.

Variable	Source of variation	df	MS	*F*	*p*
Activation	Status	1	0.925	0.690	0.407
	Sex	1	1.404	1.047	0.307
	Status x sex	1	0.867	0.647	0.422
	Error	234	1.341		
Orientation	Status	1	16.245	7.345	0.007
	Sex	1	2.033	0.919	0.339
	Status x sex	1	2.234	1.010	0.316
	Error	234	2.212		
Contact	Status	1	1.869	0.631	0.428
	Sex	1	2.716	0.917	0.339
	Status x sex	1	3.395	1.146	0.285
	Error	234	2.961		

Table 3. Summary of effects of *Trypanosoma cruzi*-infection on variables related to feeding behaviour in *Mepraia spinolai*. All comparisons were performed by two-way ANOVAs.

Variable	Source of variation	df	MS	*F*	*p*
Rostrum ejection	Status	1	9.837	4.145	0.043
	Sex	1	2.400	1.010	0.316
	Status x sex	1	4.818	2.030	0.156
	Error	251	2.373		
First bite	Status	1	5.777	3.760	0.054
	Sex	1	1.786	1.162	0.282
	Status x sex	1	4.641	3.021	0.083
	Error	251	1.536		
Feeding time	Status	1	0.199	0.869	0.352
	Sex	1	3.173	13.868	< 0.001
	Status x sex	1	0.481	2.103	0.148
	Error	251	0.229		
Number of bites	Status	1	10.652	6.188	0.014
	Sex	1	0.613	0.356	0.551
	Status x sex	1	0.138	0.080	0.777
	Error	251	1.721		
Biting rate	Status	1	8.848	15.115	< 0.001
	Sex	1	0.944	1.613	0.205
	Status x sex	1	0.009	0.015	0.902
	Error	251	0.585		
Blood intake	Status	1	4.700	39.004	< 0.001
	Sex	1	11.213	93.106	< 0.001
	Status x sex	1	0.084	0.69	0.405
	Error	251	0.120		

CAPÍTULO 3

4. Fecundity response of a trypanosome-infected kissingbug

SUMMARY

The influence of parasites on insect host fecundity has long been studied in natural and experimental conditions. Most studies, however, have evaluated the impact of parasitism on female hosts only, without consideration of the contribution of male infection for final host reproduction. In this study, I examine the influence of the protozoan *Trypanosoma cruzi* on the reproductive success of the kissingbug *Mepraia spinolai*, using an experimental design that separates the contribution of male and female infection on kissingbug reproduction. Parasitism in males and females did not affect the egg-laying rate, egg clutch size, the percentage of eggs with vitellus, the percentage of egg hatching, and the number and weight of first instar nymphs. Eggs coming from infected parents, however, were lighter than eggs coming from uninfected bugs, indicating that the protozoan affected egg quality rather than egg quantity. This finding suggests that *T. cruzi* inflict a slight reproductive cost to *M. spinolai* regardless of the sex that is infected.

1. INTRODUCTION

It widely known that parasites can reduce host fitness components. This reduction may include short-term mortality, complete or partial castration, or slight reductions in host fecundity (Minchella 1985; Ballabeni 1995; Poulin 1998). Even though the effects of parasitism are often seen, there is still a low understanding of the mechanisms involved in host fecundity reduction (Moore 2002). On the one hand, if life-history trade-offs exist, resource allocation to defense, growth and maintenance may compromise resource allocation to reproduction, and hosts may reduce reproductive effort as an adaptive strategy to tolerate parasitism (Forbes 1993; Perrin *et al.*1996). On the other hand, host fitness reduction may be largely unrelated to host adaptation, but represent a pathological side-effect of infection. In insects, most studies evaluating the effect of parasitism on host fitness have implicitly assumed that life-history trade-offs rather than pathogenicity govern host reproduction. Because strategies to avoid or tolerate parasitism are often sex-specific as a consequence of sex differences in the resource allocation trade-off between parasite defense and other components of the phenotype (Tschirren *et al.* 2003), it is possible that reproductive allocation compromises reproduction mainly in female rather than male individuals. As a result, most studies have examined the impact of parasites on the female fecundity component, without consideration of the male infection status for final reproduction (see review in Hurd 2001). However, it is known that females may receive direct benefits from mating with uninfected males if infected males have a low energy allocation to spermatophore production, provide low-quality ejaculates, or do not stimulate oviposition (Simmons

1993; Polak 1998; Fellowes *et al.* 1999; Lehmann & Lehmann 2000). Consequently, the net resource availability to host reproduction may be contingent not only to female but also to male infection status. In spite of its importance, few studies have examined the influence of both male and female infection on host reproductive success (Zuk & McKean 1996; Sheridan *et al.* 2000).

In this work, I focus on a trypanosome-kissingbug system that consists on the protozoan parasite *Trypanosoma cruzi* and its reduviid vector *Mepraia spinolai*, to inquire into the importance of sex-dependent parasitism on several host reproductive variables. More specifically, I address the following questions: (1) which are the effects, if any, of male and female infection on the reproductive success of *M. spinolai*? (2) Does *M. spinolai* females benefit from mating with uninfected males? (3) Does *M. spinolai* males benefit from mating with uninfected females?

Trypanosoma cruzi is the causative agent of Chagas disease. This heteroxenous trypanosomatid possesses a life cycle that involves several morphologically distinct stages, and infection of definitive hosts occurs by contamination with the infectious metacyclic trypomastigote stage of the flagellate (Kollien & Schaub 1997, 2000). Once inside the insect vector, the trypanosomatid multiplies and differentiates in the digestive tract. The kissingbug *Mepraia spinolai* is one of the triatomine species responsible of *Trypanosoma cruzi* transmission in arid and semiarid Chile (Lent *et al.* 1994). This strictly hematophagous and diurnal vector species distributes between 18° and 34° S and its main habitat includes stay grounds, bird nests, rock crevices, and caves (Lent & Wygodzinsky 1979). *Mepraia spinolai* requires blood of vertebrates, such as rodents, foxes, rabbits, marsupials and birds, to complete its life cycle (Sagua *et al.* 2000; Canals

et al. 2001). The development of this hemimetabolous insect includes egg, five instar nymphs, and the adult. Often one full engorgement is sufficient for moulting from one larval instar to the next (Kollien & Schaub 2000).

2. MATERIAL AND METHODS

(a) *Infected and uninfected adults*

Adult kissingbugs were obtained from a *Mepraia spinolai* colony of first laboratory generation. Along their development, all the experimental bugs were reared individually in a 3.2 cm x 3.6 cm clear plastic compartment of an 18-compartment box (11.4 cm x 20.5 cm) placed in a climatic chamber at 26° C, 65-70% relative humidity, and 14:10 h L:D cycle. Each individual compartment was provided with sandy bottom and a folded piece of paper as refuge. Experimental kissingbugs were fed from first instar nymphs throughout the fourth instar with *T. cruzi*-infected laboratory mice (C3H strain). The *T. cruzi* strain used was isolated in May 2002 from *Mepraia spinolai* bugs collected at the Reserva Nacional Las Chinchillas (31°30' S, 71°06' W, IV Region, Chile). Trypanosomes in feces and urine of field-captured insects were used to infect mice by intraperitoneal inoculation. Since 2002, the strain has been maintained by cyclical transmission across mouse generations. To ensure that nymphs fed with infected mice were truly infected, we examined the presence of *T. cruzi* in fecal samples of fifth instar nymphs by light microscopy (NIKON Diaphot-FXA), compressing 5 µl of fresh fecal drops between a slide and an 18 mm x 18 mm cover-slip. The presence of motile

parasites in 50 microscopic fields was recorded using an x400 magnification. All bugs fed with infected mice showed evidence of *T. cruzi* in their feces. Both groups of fifth instar nymphs fed on uninfected mice. All uninfected adults fed along their ontogeny on C3H uninfected mice.

(b) ***Experimental design and reproductive output***

Crossings were carried out between unmated males and females. All adults used had recently reached maturity (within two weeks). To establish the effect of *T. cruzi* on male reproductive performance, I crossed infected males with uninfected females (n = 13 crossings). To assess the effect of *T. cruzi* on female reproductive performance, I crossed infected females with uninfected males (n = 25 crossings). Control crossings included uninfected males and uninfected females (n = 21 crossings). Each couple stayed together in a 7 cm high x 6 cm diameter plastic container with a meshed-lid. Containers were labeled and provided with a folded piece of paper as refuge. Laboratory conditions were as previously described. All couples fed every three weeks on uninfected mice until engorgement. Couple survivorship and mounts were daily recorded. All couples mated during the experimental period. During the two first months of the mating period, eggs were daily removed from parental containers, counted, weighted in an analytic scale (precision: 0.1 mg) and placed in a new-labeled container. For the rest of the mating period, eggs were weekly counted and removed. The first 20 nymphs hatched from each egg-container were weighted. After that, nymphs were daily counted and removed.

I examined whether infection influenced adult weight by comparing the body weight of infected and uninfected bugs one day after reaching maturity. In addition, a separate set of female insects was dissected to extract their fresh ovaries (65 infected and 109 uninfected). All adult females had reached maturity a month before ovary extraction was performed. All weight measurements were performed in an analytic scale (precision 0.1 mg).

To examine whether reproductive output was affected by the infection status of males and females, I performed separate one-way ANOVAs by sex on the following dependent variables: (1) percentage of eggs with vitellus (empty eggshells not included) (2) egg-laying rate (measured as the number of eggs per day), (3) egg clutch size, (4) egg weight (mg), (5) percentage of egg hatching, (6) number of first instar nymphs, and (7) weight of first instar nymphs (mg). All dependent variables were checked for homogeneity of variance by using F_{max} tests and transformed to obtain normality (Sokal & Rohlf 1995). Linear regression analysis was performed between egg weight and first instar nymph weight using the mean values per crossing.

3. RESULTS

The body weight of infected males did not differ from uninfected males (mean ± 1s.e. (mg); infected: 89.23 ± 5.19; uninfected: 101.91 ± 4.66; one-way ANOVA: $F_{1,32}$ = 2.81 , p = 0.103). However, the body weight of infected females was lower than the weight of uninfected females (mean ± 1 s.e. (mg); infected: 140.91 ± 7.88; uninfected: 176.08 ± 7.82; one-way ANOVA: $F_{1,44}$ = 9.45 , p = 0.004). Because infected females

have a lower ovary weight than uninfected ones (mean ± s.e. (mg): infected = 15.59 ± 1.49; uninfected = 26.52 ± 2.32; one-way ANOVA: $F_{1,171} = 12.29, p < 0.001$), it is quite possible that lighter bugs have lighter ovaries. This assertion was partially corroborated after testing the effect of infection on ovary weight using body weight as covariate in a one-way ANCOVA. Results revealed absence of infection effect ($F_{1,171} = 0.13$, $p = 0.23$), indicating that body weight accounted for an important fraction of the variance in ovary weight.

Trypanosoma cruzi did not affect the percentage of eggs with vitellus, egg-laying rate, and egg clutch size in male and female experiments (table 1). Notwithstanding, females mated with infected males produced eggs lighter than control crossings (mean ± 1 s.e. (mg); treatment: 2.42 ± 0.01; control: 2.48 ± 0.01; table 1). Likewise, infected females mated with uninfected males produced eggs lighter than control crossings (mean ± 1 s.e. (mg); treatment: 2.41 ± 0.02; control: 2.48 ± 0.01; table 1).

The percentage of egg hatching, and the number of first instar nymphs were unaffected by *T. cruzi* in male and female treatments (table 1). The weight of nymphs coming from experimental males showed a borderline significance with control nymphs (mean ± 1 s.e. (mg); infected: 1.82 ± 0.06; uninfected: 1.89 ± 0.02; $p = 0.08$), but nymphs coming from experimental females did not differ from those coming from control crossings (table 1). Even though the effects of *T. cruzi* on egg weight did not completely translate into concomitant differences in the first instar offspring weight, results from linear regression using mean weight values per clutch, revealed a significant association between egg weight and first instar nymph weight in pooled data (b ± s.e.: 0.65 ± 0.11, $R^2 = 0.42, p < 0.001$, n = 36 clutches, figure 1).

4. DISCUSSION

Impact of Trypanosoma cruzi on host body size

Results from this study indicate that infected *M. spinolai* female size had a 1.3-fold reduction in comparison to control individuals. Infected females reached a smaller size at maturity as compared to uninfected ones, which 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, see review in Hurd 2003), trypanosomatids and their insect hosts seem to compete for metabolites and trace nutrients present in the ingested blood (Schaub 1994; Kollien & Schaub 2000). In this study, experimental kissingbugs were exposed to *T. cruzi* infection as early as the first nymphal stage, probably increasing the chance that parasites and insects compete for nutrients along host development. This potential mechanism for host size decrease requires, however, that infected hosts decrease energy allocation to growth and maintenance. Notwithstanding, studies performed on mollusks have shown exactly the opposite pattern, that is, an increase in host size due to infection (Lim & Green 1991; Ballabeni 1995; see review in Minchella 1985). These reverse alterations have been interpreted as a host physiological response that diverts resources from reproduction to somatic growth 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), I

cannot rule out the possibility that host size decrease results from fine-tuned energy allocation processes favoring insect survival over somatic growth. Hurd *et al.* (1995) have suggested that female body size can affect reproduction in two ways. First, fecundity can be limited by the number of ovarioles presents in each ovary. Second, body weight reduction may affect blood feeding and bloodmeal utilization for egg production. In this study, the reduction in body weight of *T. cruzi*-infected females translated into ovary size reduction as expected. Likewise, behavioural data on the feeding performance of *M. spinolai* indicate that feeding efficiency and size of bloodmeals decrease in *T. cruzi*-infected insects (Botto-Mahan *et al.*, in prep.).

Impact of Trypanosoma cruzi on host reproduction and the role of sex-dependent infection

Excepting for egg weight, *T. cruzi* had no overall effect on host reproduction. This lack of effect is concordant with previous findings that trypanosomatids often have a reduced effect on host reproductive rates (see review in Schaub 1992). For example, *T. cruzi*-infected *Triatoma infestans* had a slight reduction in egg-laying rate and hatching rate. Unfortunately, most previously reported results are contradictory because of the varying experimental conditions such as bloodmeal source, stage of infection, vector ageing, and trypanosome strain inoculates, which precludes useful generalizations. For example, the weakening of insects by ageing or adverse weather conditions may increase the pathogenicity of the flagellates (Schaub 1994).

Eggs coming from infected females were lighter than control eggs. Reduction in bloodmeal size due to impaired feeding behaviour of infected insects has been often invoked as an adverse effect on egg quantity and quality (Hurd *et al.* 1995). Even though infection had no effect on the egg production of *M. spinolai*, however, it is possible that maternal investment on the number of eggs compromise yolk allocation in this species. Life-history theory states that investment in offspring size is at the expense of investment into the number of offspring (Stearns 1992). This trade-off is driven by energetic, physiological, morphological and/or genetic constraints on the total reproductive output. Results from this study suggest that *T. cruzi* can represent an energetic constraint on female reproduction that translates into a constant egg production at the expense of yolk investment.

Interestingly, eggs sired by infected males showed a weight reduction in comparison to control eggs. It is known that reduction in male fitness could be caused by sexual selection and/or by the direct effect of parasite infection (Pai & Yan 2003). In this study, pre-copulatory sexual selection can be rule out because the experimental design considered crossings of one male with one female. However, post-copulatory sexual selection driven by females cannot be overlooked (Simmons 2001). For example, it has been reported that male body size influences ejaculate size and the magnitude of female postmating responses in insects (Cook 1992; Savalli & Fox 1998). On the other hand, a direct effect of infection on seminal product quality is also a plausible explanation for infection-induced egg weight reduction in male kissingbugs. For instance, seminal products have been reported to provide nutrients that have a positive influence on female

reproduction through incorporation into female somatic tissue and developing eggs (Hoelzer 1989; Rosenqvist & Johansson 1995).

In summary, even though *T. cruzi* decreased the body weight and ovary size of *M. spinolai*, most reproductive variables were unaffected by the infection. Egg weight was the only determinant of fecundity that was affected by the parasite regardless of the sex that was infected, and this effect did not translate into a concomitant reduction in first-instar nymph quality.

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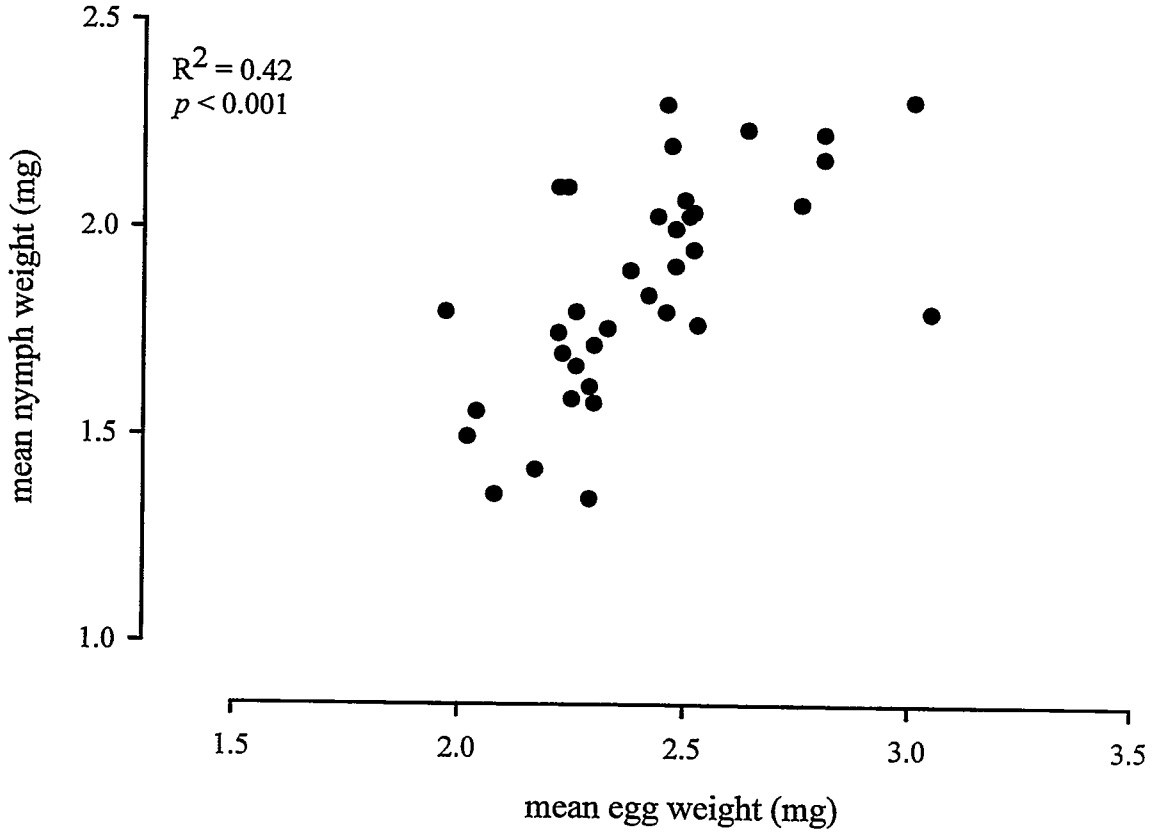
Table 1. Summary of effects of *Trypanosoma cruzi*-infection on the reproductive success of *Mepraia spinolai*. All comparisons were performed by one-way ANOVAs ([1]arcsin, [2]log, and [3]square root-transformed)

Dependent variable	*Male effect*		*Female effect*	
	F	*p*	*F*	*p*
Eggs				
[1]*Percentage with vitellus*	$F_{1,32} = 2.38$	0.133	$F_{1,44} = 1.47$	0.233
[2]*Laying rate (eggs/day)*	$F_{1,32} = 1.48$	0.233	$F_{1,44} = 2.90$	0.096
[3]*Clutch size*	$F_{1,29} = 0.71$	0.407	$F_{1,43} = 0.97$	0.331
[2]*Weight (mg)*	$F_{1,1460} = 7.36$	0.007	$F_{1,1807} = 9.04$	0.003
First instar nymphs				
[1]*Percentage of hatching*	$F_{1,32} = 1.36$	0.253	$F_{1,44} = 0.43$	0.515
[3]*Number*	$F_{1,29} = 1.33$	0.258	$F_{1,43} = 0.79$	0.380
[2]*Weight (mg)*	$F_{1,261} = 3.09$	0.080	$F_{1,345} = 2.24$	0.136

FIGURE CAPTION

Figure 1. Linear regression analysis between mean weight of eggs and first-instar nymphs from 36 crossings of *M. spinolai*.


2.5
2.0
1.5
1.0
$R^2 = 0.42$
$p < 0.001$
mean nymph weight (mg)
1.5
2.0
2.5
3.0
3.5
mean egg weight (mg)

5. DISCUSIÓN Y CONCLUSIONES FINALES

En este estudio se examinó la interacción entre el protozoo flagelado *Trypanosoma cruzi* y su vector silvestre en Chile *Mepraia spinolai* (Hemiptera; Reduviidae). Este sistema endémico permitió abordar diversos aspectos de la interacción parásito-vector hasta ahora no considerados en estudios de insectos triatominos. Se examinó de qué manera diversos rasgos del fenotipo de este organismo, tales como el tiempo de desarrollo, rasgos conductuales, y tamaño corporal inciden en la transmisión del parásito flagelado. El primer objetivo fue examinar el efecto de *T. cruzi* sobre el tiempo de desarrollo de su vector *M. spinolai*, considerando el tiempo de muda sobre una base acumulada y por estadío. Los resultados obtenidos del seguimiento de cohortes indicaron que los vectores infectados presentan menores pesos corporales y requieren más tiempo para alcanzar la madurez que los insectos control (no infectados). Se desconoce el mecanismo fisiológico que subyace a este patrón observado. Sin embargo, estudios realizados en otras asociaciones tripanosomátido-insecto indican que los interactuantes compiten por elementos trazas presentes en la sangre ingerida por los vectores, y que los insectos requerirían de un nivel crítico de metabolitos para gatillar la inducción hormonal del proceso de muda (Schaub 1992; Kollien & Schaub 2000). Una consequencia de esta demora en el tiempo de desarrollo, es que *T. cruzi* podría aumentar su probabilidad de transmisión a hospederos vertebrados definitivos. El razonamiento para esta proposición es que insectos infectados requerirían alimentarse un mayor número de veces para alcanzar la madurez (Schaub 1994) y consecuentemente transmitir el parásito vía fecas contaminadas. La modificación en

el tiempo de desarrollo y en los pesos corporales fue dependiente del estadío en cuestión. Las ninfas de los últimos estadíos fueron proporcionalmente más afectadas que aquellas de los estadíos tempranos. Esto puede ser explicado por la disminución de la tasa de crecimiento corporal de los insectos parasitados a mayor carga parasitaria (Hurd 1990), lo cual estaría acompañado con un decremento en el consumo de alimento (Thompson 1983). Una segunda explicación, no excluyente de la anterior, es que las ninfas de estadíos tardíos, cercanas a la madurez, presentan un gasto extra de energía para asignar a la producción de gametos y desarrollo de órganos reproductivos. El grado en el cual *T. cruzi* afecta el presupuesto energético de *M. spinolai*, y su influencia sobre la distribución de recursos a crecimiento y muda necesita ser evaluado en detalle en estudios de compromisos en la asignación de recursos.

Los resultados del tiempo acumulado de muda reveló que la magnitud del impacto que ejerce *T. cruzi* sobre el tiempo de muda es dependiente del sexo en consideración. Las hembras infectadas fueron proporcionalmente más afectadas que los machos infectados. En general, las estrategias para evitar o tolerar el parasitismo son específicas del sexo que se evalúe, debido a que los sexos presentan diferentes compromisos en la asignación de recursos a defensa contra parásitos y otros componentes del fenotipo (Tschirren *et al.* 2003)

El segundo objetivo del trabajo fue examinar los componentes de la conducta de alimentación de *M. spinolai* que incidían en la transmisión del parásito y determinar cuáles rasgos conductuales son modificados en presencia de *T. cruzi*. Se ha descrito que durante el período de transmisión de los parásitos, las presiones selectivas debieran favorecer a aquellos parásitos que pueden manipular a sus

vectores y así aumentar la probabilidad de transmisión hacia el hospedero definitivo (Hurd 2003). En este estudio, se encontró que conductas asociadas a la localización de hospedero definitivo, la conducta de alimentación y el patrón de deyección se modifican en presencia de *T. cruzi*. Insectos infectados requirieron menos tiempo para detectar y orientarse hacia hospederos definitivos que los insectos controles. Esto se podría deber a que insectos infectados presentan menores tamaños corporales producto de una competencia parásito-vector por nutrientes presentes en el intestino del insecto (Schaub 1992; Kollien & Schaub 2000), lo cual se traduciría en síntomas de inanición anticipados, provocando que los insectos estén más alerta frente a estímulos provenientes de potenciales hospederos. Con respecto a la conducta de alimentación, los insectos picaron más veces a sus presas y aumentaron la tasa de picada en presencia del parásito. Sabiendo que *T. cruzi* se aloja principalmente en el recto de la vinchuca, es posible descartar, en primera instancia, un bloqueo de la proboscide o el desarrollo de patologías asociadas a las glándulas salivales (Schaub 1992; Kollien & Shaub 2000), mecanismos invocados para explicar modificaciones en la conducta de alimentación de otros insectos vectores de parásitos que son transmitidos mediante inoculación salival directa (revisión en Moore 2002). Una vez más, esta conducta alterada por parte de los insectos infectados se puede deber a los mayores niveles de inanición producto de la competencia parásito-vector que se traduce en un insecto menos eficiente al momento de encontrar un vaso sanguíneo apropiado para picar y alimentarse. Por otro lado, el sexo fue determinante en los tiempos involucrados en la ingesta de sangre. Las hembras necesitaron más tiempo para alimentarse que los machos, lo cual probablemente se debe a su mayor tamaño corporal (Lent & Wygodzinsky 1979). La cantidad de sangre ingerida fue afectada

por el estatus de infección y por el sexo en consideración. Tal como ha sido indicado previamente, los insectos infectados presentaron un menor peso corporal. Al remover el efecto del tamaño corporal no se observó evidencia de un efecto del estatus de infección pero sí del sexo, lo cual sugiere que este resultado podría interpretarse como un efecto indirecto producido por una reducción del tamaño corporal de los vectores en presencia de *T. cruzi*.

Con respecto al patrón de deyección, se observó efectos del sexo y del estatus de infección. Los machos deyectaron más rápido que las hembras, debido probablemente a que éstos presentan intestinos medios y anteriores con menor capacidad de almacenaje que las hembras. Por otro lado, los insectos infectados con *T. cruzi* presentaron deyecciones precipitadas en comparación a los insectos controles. Este resultado indica que los insectos infectados presentan una mayor sincronía entre el fin del evento de picada y la emisión de fecas infectantes. Este resultado posee importantes consecuencias para el entendimiento de la transmisión parasítica, la cual ocurre precisamente al momento de liberar los tripomastigotes metacíclicos de las fecas en la cercanía de los hospederos mamíferos recién picados. Los resultados de este trabajo indican la importancia de considerar integralmente el proceso de alimentación. Los insectos infectados detectan más rápido a sus hospederos, los pican más veces implicando un mayor número de perforaciones en la piel, y deyectan en menor tiempo que los insectos controles. Todos estos antecedentes, sumado al hecho que los insectos infectados picarían numéricamente más durante su ontogenía para alcanzar la madurez, revelaría una importante presión ejercida por el parásito para ser transmitido a su hospedero definitivo.

El tercer objetivo de este trabajo fue examinar el desempeño reproductivo de *M. spinolai* en presencia de *T. cruzi*. Existe evidencia que indica que los parásitos de insectos reducen la salida reproductiva de sus hospederos (Hurd 1998; Webb & Hurd 1999). En insectos, la destrucción mecánica de las gónadas no es frecuente. Usualmente, la reducción en la producción de huevos se debe a cambios morfológicos, fisiológicos o conductuales en el fenotipo del hospedero (Webb & Hurd 1999). Un resultado de este estudio es que *T. cruzi* reduce el tamaño corporal en que ocurre la reproducción de vectores machos y hembras. Esto se debe probablemente a la reducción de nutrientes disponibles para el crecimiento del insecto (Thompson 1983; Hurd 1990). Se ha indicado que la reducción en el tamaño de las hembras puede afectar directamente la fecundidad debido a una reducción en el número de ovaríolos. Además, la reducción en peso puede reducir la ingestión de sangre y su utilización en la producción de huevos (Hurd *et al.* 1995). En este estudio, el único determinante de la fecundidad que fue sensible a la presencia de *T. cruzi*, fue el peso de los huevos provenientes de machos y hembras infectados. Otros componentes de la fecundidad tales como la producción de huevos, el peso de las ninfas y el número de ninfas producidas no fueron afectados por el estatus de infección de los parentales. Probablemente, la reducción en la cantidad de sangre ingerida por hembras infectadas se tradujo en una reasignación de los recursos dentro del item reproducción a través de mantener constante el número de huevos a expensas de la inversión en yema por huevo. En el caso de los machos infectados, el razonamiento es similar al de las hembras infectadas en término de compromisos energéticos. Los productos seminales que proveen nutrientes podrían estar empobrecidos en presencia de la infección. Como tales nutrientes pueden ser

incorporados a tejido somático de la hembra y a los huevos en desarrollo (Hoelzer 1989; Rosenqvist & Johansson 1995), es posible que la infección masculina se haya traducido en una baja contribución paternal al peso de los huevos.

En conclusión, más antecedentes son necesarios para explicar desde una perspectiva fisiológica los mecanismos que subyacen a las modificaciones en los rasgos de historia de vida y conducta del insecto vector *Mepraia spinolai* revelados en presencia de parásito *Trypanosoma cruzi*. El análisis e integración de la evidencia aquí presentada sugiere que aunque *T. cruzi* no pareciera impactar sustancialmente en la fecundidad de *M. spinolai*, los cambios inducidos en la ontogenia y conducta de alimentación del hospedero probablemente se traducen en un mayor potencial de transmisión de la infección hacia otros hospederos potenciales.

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