FEMALE MICE PREFERENCE: DOES *Trypanosoma cruzi* AFFECT MALES' SCENT?

Nathaly González Quiñónez, Adriana Pirela, Rosa De Jesús de Durán and Mariana Muñoz-Romo

SUMMARY

*Female mammals preferentially select parasite-free or disease-resistant males for mating. Thus, parasitic infections may influence mate choice. In rodents, urine and other odoriferous secretions are of paramount importance in mate attraction and selection, and females can distinguish between infected and non-infected males through their scent. In female mice, a lower preference for males infected with either endo- or ectoparasites has been observed. The main goal of this study was to examine the effect of a tropical endoparasite (*Trypanosoma cruzi*) on female mice odor preference. Adult female mice (NMRI) were individually placed in the base of a Y-maze and allowed to choose between two different odors: urine from a non-infected male and urine from an infected male. The time spent by females on each arm was recorded during 5min, as well as the number of visits to each arm where urine samples were placed. Although females invested equal times exploring the sources of odors, the number of visits of females indicated that they preferred odors from non-infected males. The results would indicate that NMRI females are able to detect parasitic infection caused by the protozoan* T. cruzi*, and presumably avoid infected males for mating.*

Introduction

Several studies suggest that parasitic infection may influence mate choice, with females preferentially selecting parasite-free or resistant males (Hamilton and Zuk, 1982; Clayton, 1991; Zuk, 1992; Ehman and Scott, 2002). The advantages accrued by a discriminating female include acquisition of parasite-resistant genes for her offspring (Hamilton and Zuk, 1982), decreased risk of contracting an infectious agent (Borgia and Collis, 1989; Able, 1996) and increased paternal care (Milinski and Bakker, 1990). Hamilton and Zuk (1982) suggested that animals should inspect both urine and fecal odors of a potential mate in addition to examining secondary sexual characteristics (Ehman and Scott, 2002).

In rodents, urine and other odor cues are of major importance in mate attraction and selection (Lenington, 1983; Egid and Brown, 1989; Potts *et al*., 1994; Brown, 1995). Both female and male mice have well-developed olfactory responses and can determine the status of conspecifics on the basis of odor cues (Lenington, 1983; Coopersmith and Lenington, 1992; Kavaliers *et al*., 1997). Studies on odor preference in rodents indicate that a wide array of infections, from gastrointestinal nematodes to viruses, can influence the odors of infected individuals, and that females demonstrate a reduced preference for odors from infected males (Penn and Potts, 1998; Klein *et al*., 1999; Willis and Poulin, 2000; Ehman and Scott, 2001, 2002).

There are many ways by which an individual's odor might signal infection. First, infection might change the composition of commensal microbes that play an important role in shaping an individual's odor. Second, infection might also trigger immunological responses that alter an individual's odor. Third, activation of the immune system probably alters the excretion of other metabolic byproducts from the endocrine system (Penn and Potts, 1998). Female laboratory mice displayed a reduced interest in, and avoidance of, the urine and other odorous secretions of males infected with either endoparasites such as the protozoan *Eimeria vermiformis* and the nematode *Heligmosomoides polygyrus*, as well as influenza virus (Kavaliers and Colwell, 1995a, b; Kavaliers *et al*., 1997, 2000, 2003; Penn *et al*., 1998; Ehman and Scott, 2001; Kavaliers *et al*., 2003).

To date, there have been no published reports exploring whether the infection with *Trypanosoma cruzi* acts as driving force in female mate choice. *T. cruzi* is a species of parasitic trypanosomes that causes the trypanosomiasis or Chagas' disease in humans and animals. One would expect female mice to select against odors of urine from infected males (*Trypanosoma cruzi*) and select preferentially odors of urine from non-infected males. The main goal of this study was to examine the effect of this tropical endoparasite on female mice odor preference.

KEYWORDS / Attraction / Mate choice / Odors / Parasites / Rodents / *Trypanosoma cruzi* /

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Nathaly González Quiñónez. Biologist, Universidad de Los Andes (ULA), Venezuela. e-mail: natygq@gmail.com

Adriana Pirela Di Vincenzo. Biology student, ULA, Venezuela. e-mail: nadriana83@hotmail.com

Rosa De Jesús de Durán. Ph.D. in Sciences, Universidad Central de Venezuela. Professor, ULA, Venezuela. e-mail: rosadj@ula.ve

Mariana Muñoz-Romo. Ph.D. in Biology (Ecology, Behavior, and Evolution), Boston University, USA. Professor, ULA, Venezuela. Address: Laboratorio de Zoología Aplicada, Facultad de Ciencias, Núcleo La Hechicera, Mérida, Venezuela. e-mail: mariana@ula.ve

PREFERENCIA DE RATONES HEMBRA: ¿AFFECTA *Trypanosoma cruzi* EL OLOR DEL MACHO?)
Nathaly González Quiñónez, Adriana Pirela, Rosa De Jesús de Durán y Mariana Muñoz-Romo

RESUMEN

*Para aparearse, las hembras de mamíferos seleccionan con preferencia machos libres de párasitos o resistentes a enfermedades, de modo que las infecciones parasitarias pueden influenciar la selección de pareja. En roedores, la orina y otras secreciones odoríferas son de gran importancia en la atracción y selección de pareja, y las hembras pueden distinguir entre machos infectados y no infectados a través de sus olores. En ratones hembra ha sido observada una menor preferencia por machos infectados ya sea con endo- o ectoparásitos. El objetivo principal de este estudio fue examinar el efecto de un endoparásito tropical (*Trypanosoma cruzi*) sobre la preferencia de olor de ratones hembra. Se utilizaron ratones (NMRI) hembra adultos, las cuales fueron colocadas individualmente en la base de un 'dispositivo en Y', para que escogieran entre dos olores diferentes: orina de machos infectados y orina de machos no infectados. El tiempo invertido por las hembras en cada uno de los compartimientos fue registrado durante 5min, así como también el número de visitas a cada compartimiento donde las muestras de orina fueron colocadas. Las hembras emplearon la misma cantidad de tiempo en explorar los olores, pero el número de visitas de las hembras indicó que ellas prefieren olores de machos no infectados (sanos). Los resultados indicarían que las hembras NMRI son capaces de detectar la infección parasitaria causada por el protozoario* T. cruzi *y presumiblemente evitarían machos infectados para aparearse.*

PREFERÊNCIA DE RATOS FEMEA: AFETA *Trypanosoma cruzi* O CHEIRO DO MACHO?)
Nathaly González Quiñónez, Adriana Pirela, Rosa De Jesús de Durán e Mariana Muñoz-Romo

RESUMO

*Para acasalar-se, as fêmeas de mamíferos selecionam preferencialmente machos livres de parasitas ou resistentes a enfermidades, de modo que as infecções parasitárias podem influenciar a seleção do par. Em roedores, a urina e outras secreções odoríferas são de grande importância na atração e seleção do par, e as fêmeas podem distinguir entre machos infectados e não infectados a través de seus cheiros. Em ratos fêmea têm sido observada uma menor preferência por machos infectados, seja com endo- ou ecto-parasitas. O objetivo principal deste estudo foi examinar o efeito de um endoparasita tropical (*Trypanosoma cruzi*) sobre a preferência de cheiro de ratos fêmea. Utilizaram-se ratos (NMRI) fêmea adultos, os quais foram colocados individualmente na base de um 'dispositivo em Y', para que escolhesem entre dois cheiros diferentes: urina de machos infectados e urina de machos não infectados. O tempo dispensado pelas fêmeas em cada um dos compartimentos foi registrado durante 5min, assim como também o número de visitas a cada compartimento onde as amostras de urina foram colocadas. As fêmeas utilizaram a mesma quantidade de tempo em explorar os cheiros, mas o número de visitas das fêmeas indicou que elas preferem cheiros de machos não infectados (sãos). Os resultados indicariam que as fêmeas NMRI são capazes de detectar a infecção parasitária causada pelo protozoário* T. cruzi *e presumivelmente evitariam machos infectados para acasalar-se.*

Materials and Methods

An odor preference test was used (Y-maze test system, see below) for examining female mice response with odors of urine from infected males (*Trypanosoma cruzi*) and of urine from non-infected males. Ten male mice (NMRI, adults, weighing 25-30g) were used as urine donors, which included non-infected males and infected males (with *T. cruzi*). Males were infected by injecting intraperitoneally an inoculum of 0.1ml containing 100,000 parasites. The urine was obtained 21 days after infection by gentle abdominal pressure, and collected using sterile swabs, stored in sterile vials at 4ºC until their use several hours later. Males were observed sick when collecting these urine samples, and died 4-5 days after collection. All individuals were maintained under uniform conditions in the same animal room.

All females used during the experiment were in estrous. The odor preferences of individual female mice were tested in a translucent Plexiglas Y-maze apparatus with two arms and three chambers (Figure 1). The stimuli (urine from non-infected male and urine from infected male with *T. cruzi* were placed in compartments in the two arms of the Y-maze device. Each female mouse (NMRI, adult, weighing 25-30g) was placed at the start box. As controls, urine samples from non-infected males were used in both arms of the Y-maze. In total, 50 female mice were used in the study (30 experimental and 20 controls). All surfaces inside and outside the Y-maze were cleaned with 95% ethanol before each trial. Powder-free nitrile gloves were used during the experiments to prevent contact with the experimenter's skin and were discarded after each trial. The location of the enclosures in the Y-maze was switched between trials. Females were never tested more than once.

In the design and operation of the Y-maze, a solid Plexiglas barrier restricted the mouse to the start box (Figure 1), avoiding its exposure to the odor cues until test time. Perforated Plexiglas barriers at the ends of the two stimulus arms prevented contact with the odor sources (Figures 1 and 2).

For all preference tests, an individual female mouse was placed in the start box of the Y-maze for 10sec, after which the solid barrier was removed allowing the mouse access to the two arms of the Y-maze. During the subsequent 5min testing period, the time spent by females on each arm and the number of visits to each arm, where urine samples were placed, were recorded (Figure 2). Only in few occasions females were observed moving inside the arm. If a female moved part-way along the arm, away from the urine and then returned, this was considered as the same visit. The time

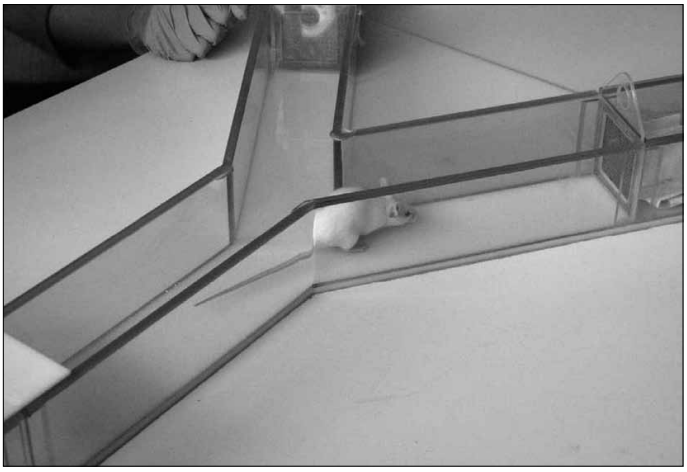

Figure 1. Y-maze device used during experiments.

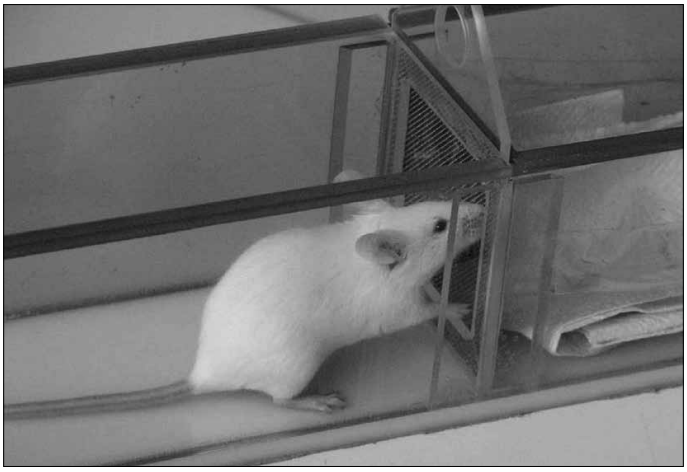

Figure 2. Female mouse sniffing a sample of urine of a male.

for testing was 5min, based on results of previous studies that established a 5min exposure time in preference tests, and showed that the results obtained were comparable to those obtained with longer exposures of 15 and 30min (Kavaliers *et al*., 1997).

A non-parametric Kruskal-Wallis (KW) test was used to examine time preferences and number of visits. All statistical tests were performed using the SPSS version 11.0.4 (SPSS Inc., Chicago, IL, USA), using α= 0.05.

Results

All females were observed sniffing continuously during trials. In terms of exploratory behavior, the number of visits by females, when presented with a choice between the odors of urine from infected (with *T. cruzi*) and non-infected males, did differ, providing evidence that the females significantly preferred (KW χ^2= 14.31, n= 60, P<0.001) the odors of non-infected males (Figure 3). When given a choice between the urinary odors from two non-infected different males (control), the number of visits by females to each Y-maze arm was not significantly different (KW χ^2= 0.136, n= 40, P=0.712; Figure 3).

Furthermore, comparison of the time invested during visits revealed that females tend to spend the same time in the arm containing the odor of infected males as in the arm containing the odor of non-infected males (KW χ^2= 1.756, n= 60, *P*=0.185; Figure 4). Females spent equal amounts of time with both odors in the Y-maze, during control trials (KW χ^2= 0.514, n= 40, *P*=0.473; Figure 4).

Discussion

The results show, for the first time, that female mice can distinguish the odors of non-infected male mice from those of males infected with *Trypanosoma cruzi*, a tropical endoparasite.

Based on the number of visits of females to the arms containing the odors of infected and non-infected males, the results indicate that female mice find the urinary odors of non-infected males more attractive than those of infected males. The fact that females spent equal amounts of time in both arms (with odors from infected and non-infected males) could be related to the fact that once in presence of an odor cue, females spent a specific amount of time exploring the source of odor. This result is consistent with other studies that indicated that time preferences do not correlate with ultimate mate choice (Bilbo *et al*., 1999; Ehman and Scott, 2002).

In agreement with the present findings, previous studies demonstrated that female rodents can distinguish between, and avoid, the odors of males infected with other endoparasites (Kavaliers and Colwell, 1995a,b; Kavaliers *et al*., 1997, 2000, 2003; Penn *et al*., 1998; Klein *et al*., 1999; Willis and Poulin, 2000; Ehman and Scott, 2001). Substantial evidence exists that mice and rats can distinguish urinary odors associated with differences in the major histocompatibility complex (MHC; Kavaliers *et al*., 1997). Rodents may utilize recognition cues provided by major urinary proteins. Female mice use MHC mediated or related odors for both kin recognition and mating preferences (Yamazaki *et al*., 1976; Egid and Brown, 1989; Eklund *et al*., 1991; Potts, *et al*., 1994; Brown, 1995).

There are advantages for a female to be able to discriminate between non-infected and infected potential mates (Penn and Potts 1998). The benefit of choosing non-infected

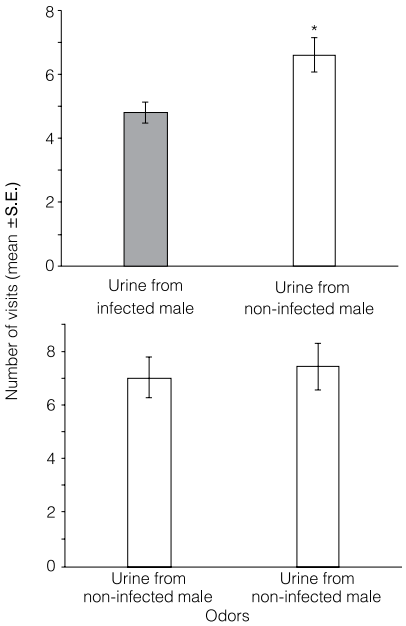

Figure 3. Number of visits of females to both arms of the Y-maze device, during experimental (above) and control (below) trials.

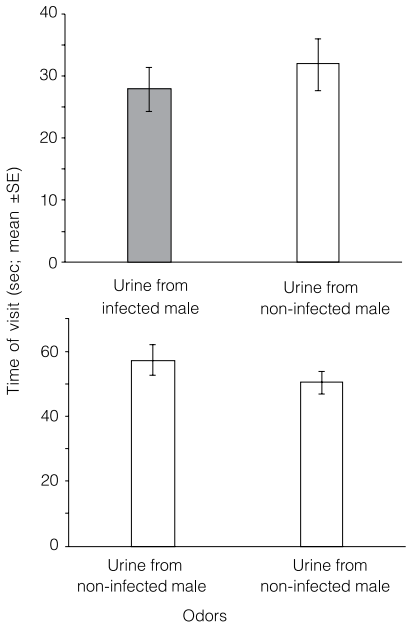

Figure 4. Time of visit of females to both arms of the Y-maze device, during experimental (above) and control (below) trials.

partners would most likely be related to a reduction in the risk of transmission, but also to acquisition of parasite-resistant genes for their offspring. This implies a parasite mediated sexual selection and assumes that a genetic advantage is conferred by the 'resistant' non-infected male, and that parasite resistance is heritable (Penn and Potts, 1998; Ehman and Scott, 2002).

The use of a Y-maze does not test mating preferences directly, but instead it tests social interest or preference (Lenington, 1983). Results of previous studies have shown that preferences in a Y-maze are consistent with mating preferences (Kavaliers *et al*., 2003). Female preferences in odor choice tests are considered to be consistent with mating preference, and are suggested to give reliable indications of mate choice (Krackow and Matuschak, 1991; Coopersmith and Lenington, 1992; Kavaliers *et al*., 1997). Thus, it could be said that female mice likely discriminate males infected with *Trypanosoma cruzi* from non-infected males during mate choice. Odors appear to serve as a salient and direct cue of an individual's current health status and could play a key role in mate choice decisions (Ehman and Scott, 2002; Muñoz-Romo *et al*., 2011).

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