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Influenza Infection Neutralizes the Attractiveness of Male Odour to Female Mice (Mus musculus)

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Abstract

This study aimed to determine if female house mice, Mus musculus domesticus, are able to assess a male’s infection status from odour cues. We collected urine from male mice before, during, and after they were experimentally infected with influenza, a respiratory virus. Females spent more time investigating urine collected from males while they were uninfected than when they were infected. Also 70% of females released into a large enclosure preferred to nest in boxes containing urine collected from uninfected rather than infected males. This is the first evidence that mice can discriminate virally infected individuals through chemical signals and the first evidence that infection causes odour changes in the urine. To determine if the odour of infected males is repellent, we presented females with urine samples and neutral water blanks. Normal urine collected from uninfected males was more attractive, whereas urine collected during infection was as attractive as water. This indicates that rather than being aversive, influenza infection abolishes the attractiveness of a male’s odour. A similar effect also occurs when male mice are infected with coccidian gut parasites (Kavaliers & Colwell 1995, Proc. R. Soc. Lond. 261B, 31–35). One proximate reason for the neutralization of the attractiveness of a male’s odour may be a decrease in serum androgen concentrations during infection.

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Introduction

Hamilton & Zuk (1982) suggested that females use the condition of a male’s secondary sexual displays to assess his parasite load. They also suggested that females that avoid mating with infected males will increase the disease resistance of their offspring (the Hamilton & Zuk hypothesis). Females that avoid infected males will also benefit directly by reducing their risk of contracting infectious diseases (the transmission-avoidance hypothesis) (Borgia 1986; Able 1996). Numerous studies indicate that females can assess a male’s parasite load by the condition of his secondary sex characters (Read 1988; Endler & Lyles 1989; Pomiankowski 1989; Möller 1990; Clayton 1991; Able 1996). However, these studies have focused on visual and acoustic cues and have ignored chemical signals. This is an unfortunate oversight because chemical signals are used by males in a diversity of species.
to attract females (Brown 1979; Agosta 1992) and the mechanisms controlling chemical communication—scent glands, marking behaviour, odorant chemistry, and olfactory organs—are often sexually dimorphic (Blaustein 1981; Dorries 1992). Do a male’s chemical signals honestly convey information about his infection status?

Several researchers have suggested that a male’s odour is used by his rivals as well as potential mates to assess his health and infection status. Fisher (1915) suggested that human individuals who find halitosis unattractive would have a selective advantage because halitosis is associated with various diseases. McCulloch (1969) proposed that females investigate the scent marks of potential mates to assess their physical condition through the metabolic by-product in their urine. Similarly, Coblenz (1976) suggested that subordinate males investigate the metabolic by-products in the scent marks of dominant males for evidence of declines in health. He pointed out that if males attempt to hide their poor condition by terminating scent marking, then their behaviour could also be used as an indicator of poor health. Hamilton & Zuk (1982) suggested that females, like physicians, should examine urine and faecal samples from potential mates to assess their infection status. In fact physicians and veterinarians have long used odour cues, including halitosis, flatulence, body, urine and faecal odour, as indicators of disease (Liddell 1976; Brown 1995). This strongly suggests that a male’s chemical signals should honestly reflect his infection status to potential mates.

Male house mice mark their territories with urine; females are attracted to the odour of these marks (Rowe 1970; Hurst 1990), although it has never been clear why. Kavaliers & Colwell (1992) found that female mice exposed to the odour of males infected with coccidian protozoans, *Eimeria vermiformis*, increase their pain thresholds through endogenous opioid- and non-opioid-mediated analgesia. This finding indicates that female mice can discriminate infected and uninfected males by their odour. Subsequent studies found that females prefer the odour of uninfected males to those infected with either coccidia (Kavaliers & Colwell 1995a) or nematode worms, *Heligmosomoides polygyrus* (Kavaliers & Colwell 1995b). The source of the odour cue—urine, faeces, saliva, or scent gland secretions—that the females used to distinguish infected males was unclear from these studies, however. The researchers collected the odour samples from males by placing filter paper in their cages. Thus, females inspecting the filter paper may have simply detected components of these gut parasites shed in the male’s faeces rather than using conditional, secondary sexual odour signals as cues of infection (Able 1996).

To determine if female house mice can use odour cues from males to discriminate their infection status, we performed an odour-preference experiment using wild-derived female mice and urine collected from male mice experimentally infected with influenza, a respiratory virus. Females that avoid mating with infected males could increase the resistance of their offspring because mice show genetic (MHC) resistance against the influenza virus (Brown et al. 1988). We hypothesized that female mice would prefer the urine odour of a male when he is uninfected to the odour of the same male’s urine collected while he was infected. We found that females were able to distinguish the odour of infected males, but rather than being
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aversive, influenza infection neutralized the attractiveness of a male’s urine odour to females.

Methods

We conducted this experiment from August 1995 to July 1996, at the University of Florida, Gainesville, Florida, USA.

Subjects

The subjects in our experiments were 80 wild-derived, age-matched female mice, *Mus musculus domesticus*, that were colony reared (Potts et al. 1991). The female’s oestrous condition was determined before each trial by examining a vaginal smear with a phase-contrast microscope, and females in late pro-oestrus, which are the most sexually receptive, were used preferentially. Odour-donors were age-matched male mice (Balb/c) obtained from Jackson Laboratories. Male mice were housed under specific pathogen-free conditions (22 ± 2°C, 12:12 light:dark cycle, light on a 0800 h) in 13 x 18 x 29 cm acrylic cages. Food and water were available ad libitum. The male mice were kept in separate cages to prevent fighting and the formation of dominance relations, which affect the attractiveness of their odour (Jones & Nowell 1974). We collected urine from males by palpating their bladders once per day, before infection, during infection, and post-infection. The urine samples were initially stored at -80°C and later at -20°C before testing.

Influenza Infection

*Mouse influenza*. The influenza virus is a respiratory virus that infects a variety of birds and mammals (Webster et al. 1992). Influenza is not known to be a natural pathogen of house mice, but it is not unnatural or uncommon for pathogens to move into new host species (Levins et al. 1994). When mice under anaesthesia are infected with influenza via an intranasal route, this results in pneumonia (total respiratory tract infection) (Renegar 1992). The influenza A/PR/8/34 strain is a 1934 Puerto Rican isolate. Presumably owing to the many generations of passages through both mice and chicken eggs (influenza is traditionally grown in eggs), A/PR/8/34 represents a minimal threat to humans (Federal Register, 1994 Guidelines for Research Involving Recombinant DNA Molecules, NIH Guidelines). This mouse-adapted virus is not easily transmitted among mice and usually requires direct introduction of virus into the mouse respiratory tract to achieve infection.

Infections. The 15 male mice used as odour donors received 15 μl of the influenza A/PR/8/34 viral strain administered intranasally. All inoculations were conducted after the mice received 0.1 mg/g of weight of sodium pentobarbitol. This anaesthesia ensures that a total respiratory tract infection rather than an upper respiratory tract infection is achieved. The weight of the mice was recorded on the initial day of infection and daily thereafter for 14 days, which represents
the period of time necessary to recover from the infection. A 10% weight loss was used to verify that each experimental mouse was infected.

**Odour-preference Assays**

Female mice were individually tested for their preference for a male's urine, collected while the male was infected and uninfected. Females were simultaneously presented with two urine samples, one collected during infection and another when the male was uninfected, either before (preinfection) or after infection (post-infection). Using urine from the same male enabled us to control for variation in odour among males. We used 20 different females for each of the odour-preference assays.

The odour-preference test was conducted using an experimental apparatus constructed from three (0.06 m²) clear plastic cages connected with clear vinyl tubing (5 cm diameter). The middle start box was empty while the two end boxes contained equal amounts of food, water, and bedding. Two 50 μl (coded) urine samples were placed onto two different 5-cm glass dishes positioned in the two end boxes. After 5 min, we released a female into the start box and recorded the urine sample she investigated first (initial preference), the amount of time spent investigating the urine sample (sniff-bout duration), and the number of investigations (number of sniff bouts) continuously over a period of 30 min. A sniff bout was defined as the occurrences in which a subject crossed the perimeter of a glass dish with her nose. We conducted a similar odour-preference test using urine samples from infected males versus water blanks. We videotaped the behaviour of the mice during these tests so that we could more accurately quantify their behaviour. The settlement-preference test was conducted immediately after the odour-preference test by releasing the female being tested into a large (7.5 m²) enclosure with the two end boxes containing the urine samples (thus, each female was tested twice, once in an odour-preference test and once in a settlement-preference test). We recorded the location of the mouse 24 h later (the females were invariably in the nest boxes during the day). All of the urine samples were coded earlier so that the observers were unaware of the type of sample being presented.

**Statistical Analyses**

We used non-parametric tests to analyse the cumulative responses of females during 30-min tests because they were not normally distributed nor had equal variances.

**Results**

When females were presented with urine samples collected from a male during infection versus post-infection, the females spent significantly more time investigating the post-infection urine (sniff-bout duration; Wilcoxon signed rank; n = 20;
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There was no difference in the female’s initial preference (13/20 preferred uninfected) or the number of sniff bouts (Wilcoxon signed rank; n = 20; p = 0.32) between the infection and post-infection samples. After 24 h, females were more likely to be found in the nest box containing the post-infection urine sample, a pattern that was marginally significant (binomial test; n = 20; p = 0.058).

Given the choice between urine samples collected from a male during infection versus pre-infection, the females spent significantly more time investigating the pre-infection urine samples (Wilcoxon signed rank; n = 20; p = 0.042; Fig. 1b). There was no significant difference in their initial preference (9/20 preferred uninfected) or the number of sniff bouts (Wilcoxon signed rank; n = 20; p = 0.12) between the infection and pre-infection samples. Females were more likely to be found in the nest box containing the pre-infection urine samples after 24 h (binomial

Fig. 1: a. Mean duration of female investigation of urine samples collected from male mice before infection (pre-infection) versus during infection for each of three 10-min intervals ($X \pm SE$; cumulative mean sniff-bout duration pre-infected = 25.2 ± 2.8 s vs. infected = 18.9 ± 2.8 s; Wilcoxon signed rank; n = 20; p = 0.015). b. Female investigation of urine samples collected from male mice during infection versus after infection (post-infection) for each of three, 10-min intervals ($X \pm SE$; cumulative mean sniff-bout duration infected = 20.7 ± 2.1 s vs. post-infected = 32.0 ± 3.6 s; Wilcoxon signed rank; n = 20; p = 0.042)
More females (28/40) settled in nest boxes containing urine collected while males were uninfected (pre- and post-infection combined) than infected (binomial test; n = 40; p = 0.01).

When females were presented with urine samples from normal, uninfected males (pre-infection) versus water blanks, they spent significantly more time investigating the normal urine (Wilcoxon signed rank; n = 20; p = 0.002; Fig. 2). Females directed a greater but non-significant number of sniff bouts towards the urine than the water samples (Wilcoxon signed rank; n = 20; p = 0.07). When presented with urine samples from infected males versus water blanks, there was no significant difference in sniff-bout duration (Wilcoxon signed rank; n = 20; p = 0.23; Fig. 3) or the number of sniff bouts (Wilcoxon signed rank; n = 20; p = 0.38).

**Discussion**

Females spent more time investigating the odour of a male collected while he was uninfected, both before and after infection, than odour collected while he was infected. This result supports the hypothesis that infection reduces a male’s attractiveness to females. Because the comparisons were made on the same individual male, these odour preferences were not due to individual variation among males. And because females preferred the odour of males during pre- and post-infection, this result cannot be due to a female preference for older or younger males. Females showed no initial preference, but this may have been due either to odour samples being placed too far apart (1.5 m) to be detected at the onset of the experiment, or to random exploratory behaviour in a novel environment.

We also found that 70% of the females settled in nest boxes containing urine from uninfected males in the 24 h settlement assay. This settling bias may reflect a

![Graph](image)

*Fig. 2: Female investigation of urine samples collected from normal uninfected (pre-infected) male mice versus water blanks for three 10-min intervals (X ± SE; cumulative mean sniff-bout duration uninfected = 23.6 ± 2.9 s vs. water = 10.9 ± 2.2 s; Wilcoxon signed rank; n = 20; p = 0.002)*
Fig. 3: Female investigation of urine samples collected from infected male mice versus water blanks for three 10-min intervals (\( \overline{X} \pm SE \): cumulative mean sniff-bout duration infected = 8.3 ± 1.1 s vs. water = 8.2 ± 1.2 s; Wilcoxon signed rank; n = 20; p = 0.38)

social or mating preference. A male's odour appears to influence where female house mice settle, and once they settle on a male's territory, females usually mate with the dominant, territorial male (Potts et al. 1991, pers. obser.). Female mice also mate with neighbouring territorial males, and their extra-pair mating preferences appear to be influenced by a male's odour (Potts et al. 1991). The results of our 24-h settlement preference test provide stronger evidence for odour-mediated mating and social preferences than brief 3–15 min preference tests (Kavaliers & Colwell 1995a,b), however, these possibilities remain untested.

A female's oestrous condition had no effect on her preferences in our study. We examined oestrous condition because some studies have found odour and social preferences in female mice only during oestrus (Egid & Brown 1989; Coopersmith & Lenington 1992). However, not all studies find that a female's preferences are contingent upon her oestrous condition. For example, Kavaliers & Colwell (1995a) found odour preferences in female mice although most individuals were probably not in oestrus (they did not check). It might seem that the lack of oestrous effect in these studies is inconsistent with the hypothesis that these odour preferences have a sexual function. However, this prediction is not so clear. Females should examine the health of prospective mates throughout their reproductive cycle, and they may be less choosy in oestrus when they have a closing window of mating opportunities. This point raises a potential flaw with mate-choice studies that failed to detect any mating preferences in singly housed or hormonally manipulated individuals.

The females in our study were attracted to normal urine over water, but they did not differentiate between the odour of infected males and water samples. This indicates that the odour of uninfected males is attractive, but the odour of infected males simply loses its attractiveness rather than being aversive. This neutralization effect has also been found when male mice are infected with coccidian parasites (Kavaliers & Colwell 1995a). If females were repelled by the odour of infected
males, then this would support the hypothesis that the opioid-mediated analgesic response, that occurs when females are exposed to the odour of infected males, indicates stress (Kavaliers & Colwell 1992, 1995a,b). An indifference to the odour of infected males, however, does not indicate stress, nor would it provide a particularly effective mechanism for avoiding disease transmission (Borgia 1986; Able 1996). Therefore, we suggest that females lose their attraction to the odour of infected males to avoid mating with such males. This hypothesis predicts that females increase their levels of endogenous opioids when exposed to the odour of infected males to inhibit sexual receptivity (Sirinathsinghji 1984; Bednar et al. 1987; Hammer et al. 1994). The stress and sexual inhibition hypotheses are not mutually exclusive because a stress response may also inhibit sexual receptivity.

How does infection neutralize the attractiveness of a male’s odour? It is likely that the pathological effects of infection, hormonal changes (glucocorticoids), and immunological activation would all alter an infected individual’s odour. Major histocompatibility complex (MHC) genes control both immunological responses and individual odour in mice (Penn & Potts 1998) and their expression is upregulated during an immune response; therefore it has been suggested that the MHC plays a role in altering the odour of infected mice (Kavaliers & Colwell 1995a,b). However, if female mice are attending to chemical cues from the immune system, then why do they not avoid the odour of infected males? Their behaviour suggests that some odorant is missing from the urine of infected males that is normally attractive. Androgen levels generally decline during infection (Isserhoff et al. 1986; Mutayoba et al. 1994; Honour et al. 1995; Dunlap & Schall 1995), and lowered androgens apparently decrease the attractiveness of a male’s odour to females (Taylor et al. 1980; Ferkin et al. 1994). It is unclear why androgens decline during infection, but this response may ameliorate the potential immunosuppressive effects of these steroid hormones (Folstad & Karter 1992) or provide a mechanism to reallocate energy and resources from reproductive into immunological functions (Wedekind & Folstad 1994).

It has been argued that for sexual signals to be honest indicators of quality, they must be costly, otherwise individuals could simply cheat and signal their quality dishonestly (the handicap theory) (Zahavi 1975; Grafen 1990). A male’s scent-marking behaviour is costly in terms of time and energy (Gosling 1982) and attracts predators (Viitala et al. 1995). However, a male’s infection status might be signalled honestly through his odour, without any particular handicap, because producing a normal, healthy odour may be virtually impossible or too costly for infected males to disguise. Chemical signals provide a direct channel into an individual’s physiological status, therefore, they may provide honest indicators of a male’s infection status without a handicap. It remains to be seen if infection alters a male’s mating and reproductive success in house mice.

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**Literature Cited**


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