The Journal of Experimental Biology 216, 120-126
© 2013. Published by The Company of Biologists Ltd
doi:10.1242/jeb.072983

REVIEW

Parasite-augmented mate choice and reduction in innate fear in rats infected by *Toxoplasma gondii*

Ajai Vyas

School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore, 637551

avyas@ntu.edu.sg

Summary

Typically, female rats demonstrate clear mate choice. Mate preference is driven by the evolutionary need to choose males with heritable parasite resistance and to prevent the transmission of contagious diseases during mating. Thus, females detect and avoid parasitized males. Over evolutionary time scales, parasite-free males plausibly evolve to advertise their status. This arrangement between males and females is obviously detrimental to parasites, especially for sexually transmitted parasites. Yet *Toxoplasma gondii*, a sexually transmitted parasite, gets around this obstacle by manipulating mate choice of uninfected females. Males infected with this parasite become more attractive to uninfected females. The ability of *T. gondii* to not only advantageously alter the behavior and physiology of its host but also secondarily alter the behavior of uninfected females presents a striking example of the 'extended phenotype' of parasites. *Toxoplasma gondii* also abolishes the innate fear response of rats to cat odor; this likely increases parasite transmission through the trophic route. It is plausible that these two manipulations are not two distinct phenotypes, but are rather part of a single pattern built around testosterone-mediated interplay between mate choice, parasitism and predation.

Key words: anti-predator behavior, behavioral manipulation, major urinary proteins, parasitism, sexual selection, sexual transmission, trophic transmission, testosterone.

Received 25 March 2012; Accepted 12 July 2012

Introduction

Parasitism is usually thought of as a relationship of exploitation. A parasite exploits its host to derive benefits of food, to obtain shelter and to gain access to a subsequent new host. Often such exploitation takes the form of behavioral manipulation, whereby the parasite alters the behavior of the infected host in order to increase its own transmission efficiency. For example, many parasites have evolved a two-host life cycle that alternates between a prey species and a predator species, taking advantage of natural predation to gain entry into new hosts. In several of these cases, the parasite changes the behavior or appearance of the infected host in ways that are likely to increase rates of predation (Moore, 2000). The ability of the parasite to alter the behavior of its host presents a striking example of the 'extended phenotype', where the genotype of the parasite manifests its phenotype outside the physical confines of its body (Dawkins, 1982).

The protozoan parasite *Toxoplasma gondii* has been extensively studied in the context of parasitic change in host behavior. *Toxoplasma gondii* undergoes a two-stage life cycle that alternates between its definitive host (felines) and intermediate hosts (almost all warm-blooded organisms, including rats). Rats retain an evolutionarily conserved innate fear of cat odors (Apfelbach et al., 2005), which is detrimental to the parasite because it relies on predation to gain entry into definitive host. Thus, *T. gondii* alters the behavior of infected rats by abolishing innate fear, creating instead a 'fatal attraction' (Berdoy et al., 2000). Very interestingly, the behavioral effects of *T. gondii* infection are fairly specific to innate fear, leaving many other related or energetically costly behaviors intact (Berdoy et al., 1995; Lamberton et al., 2008; Vyas et al., 2007a). This specificity argues that effects of infection are a specific behavioral manipulation, rather than arising from generic sickness.

Toxoplasma gondii is also transmitted sexually in sheep, dogs and rats (Arantes et al., 2009; Dass et al., 2011; de Moraes et al., 2010). For example, the parasite invades rat testes and is ejaculated along with semen, infecting females and resultant pups. In another leap of manipulative brilliance, the parasite alters mate choice in rats whereby infected males are deemed more attractive by sexually receptive females (Dass et al., 2011). This change in the behavior of uninfected females is likely to increase sexual transmission of *T. gondii*. But there is a bit of evolutionary depravity in these events. In general, females tend to detect and avoid males infected with an array of bacteria, protozoan, lice and nematodes (Kavaliers et al., 2006; Kavaliers et al., 2005a). The odor of parasitized males is stressful for females; for example, it initiates an analgesic response in female mice (Kavaliers and Colwell, 1992). Female avoidance of parasitized males likely reflects the cost of mating with a male of poor genetic legacy and/or risk of direct transmission of parasites (Able, 1996; Hamilton and Zuk, 1982). *Toxoplasma gondii* infection results in inversion of this innate aversion of females, instead instituting an attraction to parasitized males.

Thus, *T. gondii*-infected rats demonstrate two distinct behavioral manipulations in fear response and in mate choice. In this Review, I will first discuss the relationship between parasites, predation and mate choice. I will then discuss plausible mediators of changes in mate choice in infected rats. Subsequently, I will discuss the inter-relationship between changes in mate choice and changes in fear response.

Mate choice, parasitism and predation

In many species, females provide greater parental care and investment than males. This leads to a biased operational sex ratio, manifested as more sexually receptive males than females. As a direct result of such bias, males compete between themselves for access to females and females have the opportunity to choose from many suitors. This leads to sexual selection, resulting in evolution of male traits that are useful in fighting competitor males (intra-sexual selection) and influencing mate choice of females (inter-sexual selection) (Andersson, 1994; Andersson and Simmons, 2006). The intra-sexual selection often results in evolution of conspicuous male phenotypes that aim to advertise and attract females (e.g. bright plumage in birds).

From the perspective of this review, mechanisms driving mate choice in rats are of special interest. Several mechanisms have been proposed to explain preference of females for certain male traits.

It is possible that females choose males that provide a direct and immediate benefit to the female and/or their progeny (Møller and Jennions, 2001). Relevant examples include parental care or nuptial gifts (food or material tokens transferred by male during courtship or mating). Male rats provide neither parental care nor nuptial gifts. However, it is possible that female rats choose males in order to reduce the chances of direct associative transmission of parasites or diseases (Able, 1996). That will constitute a mate choice driven by direct benefit.

It is also possible that mate choice is based on indirect benefits that hinge on the greater fitness manifested by a female's offspring if she is mated with a preferred male. There are two main categories of models that have been proposed to explain indirect benefits (Andersson, 1994; Jones and Ratterman, 2009). In the first instance, preferred male traits evolve through a circular process whereby the female prefers a male trait, and she then goes on to produce male offspring with the preferred trait and female offspring with the preference of that trait. This results in a Fisherian runaway selection until the advantage of preferred traits becomes balanced by natural selection for its cost (Fisher, 1915; Hall et al., 2000). This view does not require a positive relationship between the preferred trait and male genetic quality. In the second instance, mate choice evolves because males with preferred traits produce offspring with greater viability (Andersson, 1994; Jones and Ratterman, 2009; Smith, 1991). In other words, preferred traits serve as proxies for the genetic legacy that a male can impart to the offspring. This legacy can be in the form of, among others, better defenses against parasites and/or diseases, increased ability to produce heavier muscle mass to fight competitors, or increased ability to combat oxidative stress. These 'good gene' models suffer a serious problem in that continual selection should eliminate any heritable variation in the preferred trait. Thus if female rats prefer males with a particular pheromone, males possessing that pheromone will leave more offspring and eventually all males will have the preferred pheromone. In order to reconcile this problem, it has been suggested that preferred traits are selected because they are co-related with 'good genes' that provide heritable resistance to the parasites (Endler and Lyles, 1989; Getty, 2002; Hamilton and Zuk, 1982; John, 1997; Read, 1988). Because of constant co-evolutionary cycles between host and parasite, genes for resistance never reach equilibrium, and thus can sustain continual variation and selection. It is not yet possible to ascertain whether either of these scenarios (or both simultaneously) have played a role in mate choice of rats.

Yet, it is interesting that female rodents detect and avoid males infected with a variety of parasites, including ectoparasites, protozoans, nematodes and bacteria (Ehman and Scott, 2002; Kavaliers et al., 2006; Kavaliers et al., 2005a; Kavaliers et al., 2005b; Kavaliers and Colwell, 1992; Kavaliers and Colwell, 1995; Kavaliers et al., 1998a; Kavaliers et al., 1998b; Kavaliers et al., 2003; Klein and Nelson, 1999; Willis and Poulin, 2000; Zala et al., 2008). Female rodents have evolved dedicated neural machinery that serves to detect odors emanating from infected individuals and to generate an aversive response. Thus female mate choice is influenced by the parasitic status of the male. It is difficult to hypothesize whether aversion to parasitized males evolved because of an evolutionary need of females to avoid direct associative infection or to provide for heritable resistance to the parasites.

Rat females show mate preference even when they have access to only male urine marks or soiled bedding (Dass et al., 2011; Taylor et al., 1982). The vomeronasal system is likely to be important for the determination of mate quality, in synergy with the main olfactory system (Baum and Kelliher, 2009; Tirindelli et al., 2009). This system emanates from the vomeronasal organ and contains several transmembrane receptors that bind to odorants. These receptors include members of the vomeronasal receptor family and formyl peptide receptors (reviewed in Baum and Kelliher, 2009; Tirindelli et al., 2009). The formyl peptide recptors are postulated to play an important role in the determination of diseased states (Rivière et al., 2009). Olfactory information emanating from the vomeronasal system is relayed *via* the accessory olfactory bulb to the medial amygdala and hypothalamic nuclei that mediate behavioral and physiological responses (Baum and Kelliher, 2009). Nonapeptide oxytocin plays an important part in recognizing pathological states during the processing of olfactory information in the medial amygdala (Kavaliers and Choleris, 2011). Hence, sexual signals in rats appear to be olfactory in nature. Rat urine contains a high amount of proteins, including major urinary proteins of the lipocalin family (Beynon et al., 2008). The major urinary proteins are testosterone dependent (Roy et al., 1987; Soares Vda et al., 1987). Thus, it can be speculated based on the available evidence that major urinary proteins serve as sexual signals in rats.

Thus, sexually selected traits might evolve in order to advertise heritable capacity to ward off parasites and/or decrease the risk of direct transmission of parasites during sexual encounters (Able, 1996; Hamilton and Zuk, 1982). At the crux of this interpretation is the idea that, in many species, sexually selected traits are expensive in terms of metabolism and opportunity, setting up a trade-off. The cost of sustaining these traits confers a form of honesty whereby only fit males can afford to display them.

Thus, the costs of sexual display are important in maintaining honesty of mate choice. What are the costs? First, sexually selected traits are, by necessity, conspicuous; they evolve to communicate and advertise. One of the collateral costs of such traits is that predators find it easier to locate conspicuous males, thus setting up a trade-off (Zuk and Kolluru, 1998). Second, expression of sexually selected traits requires testosterone in many species, including rats (Ardia et al., 2010; Bonisoli-Alquati et al., 2011; Bottoni et al., 1993; Folstad and Karter, 1992; Longpre and Katz, 2011; Roney et al., 2006; Taylor et al., 1982; Wingfield et al., 1997; Zala et al., 2008). Testosterone is a steroid produced in the testes, essential to create an immune-privileged environment for developing sperm. Over evolutionary time scales, testosterone has been co-opted to mediate a trade-off between sexually selected traits and investment in the immune system. The immune-suppressive cost of testosterone confers

honesty, allowing only fit males to display the sexually selected traits. Further, in addition to its role in organizing sexual behaviors, testosterone can cross the blood–brain barrier and reduces the fear response (Aikey et al., 2002; Hermans et al., 2006; Kavaliers et al., 2001; King et al., 2005), which might further increase the predation rates on high-testosterone males.

Manipulation of mate choice

Exploitation of sexual signals by parasites is fairly common in animals (Haynes and Yeargan, 1999; Zuk and Kolluru, 1998). Sexual signals are usually an open broadcast system, liable to reception by both the intended audience (females) and an unintended audience (parasites). Thus many parasites use host sexual signals to locate a new host. Such exploitation can lead to robust selection pressure on the host. For example, male crickets use acoustic signals to attract females. In the Hawaiian Islands, where the range of distribution of crickets overlaps with that of a parasitoid fly, the parasitoid uses the sound of calling males to locate its host and lay eggs. The developing parasitoid larva burrows into the male and consumes it from inside-out. Because calling males are disproportionately targeted by the parasitoid, natural selection has recently resulted in emergence of a novel phenotype on the island of Kauai, whereby males no longer produce calling songs but become satellites to the few remaining calling males (Zuk et al., 2006). This, along with many more examples, demonstrates that parasites are a very significant cost to host mate choice, representing a robust selection force to constraint host sexual selection. Usually this exploitation takes the form of parasites using sexual signals or mimicking sexual signals to locate their host; parasites in these cases do not manipulate mate choice directly.

The rich interplay between host sexual selection and parasites posits a very interesting and as yet under-explored possibility. From the perspective of the parasite, it is highly beneficial to successfully manipulate the mate choice of the host. For example, a sexually transmitted parasite will benefit directly if it could augment the attractiveness of the host male. This is also true for parasites that depend on close physical proximity of hosts for dispersal, for in many species the sexual encounter requires the male and female to be in close contact. Related to that, host itself benefits from infection in this situation, as parasitic manipulation enhances its odds of reproducing with more females. Relatively less obvious is the possibility that a trophically transmitted parasite might also benefit from augmented host mate choice, even if the parasite does not depend on sexual intercourse for transmission (Lafferty, 1999). More attractive infected males will increase the probability of parasite transmission, because sexually displaying males are easier to locate by predators and the testosterone that sustains the sexual display also reduces fear. Moreover, manipulation of host mate choice will result in the host recouping some of the cost of parasitism, thus blunting selection pressure from the co-evolving host. If a parasite relies on both sexual/vertical and trophic routes, the benefits of manipulation are additive. The possibility of such ‘love-potion’ parasitism has been discussed sporadically (Lafferty, 1999), but has not been experimentally tested. We discuss presently available experimental evidence for this phenomenon using the *T. gondii* rat association.

In this association, *T. gondii* is trophically transmitted when an infected rat is eaten by a cat. Recent evidence suggests that this parasite is also transmitted during sexual intercourse. For example, *T. gondii* is transferred along with the semen of infected male dogs, resulting in infection of the mated female and sired progeny (Arantes et al., 2009). Sexual transmission has also been noted in rabbits (Liu et al., 2006). Similarly, artificial insemination of sheep using semen spiked with *T. gondii* results in transmission of the parasite to females (de Moraes et al., 2010). Recently we have demonstrated successful transmission of the parasite from infected rats to mated females and resultant pups (Dass et al., 2011). Dual transmission routes reflect an additive advantage of manipulating host mate choice for this parasite. We tested this possibility in laboratory conditions by quantifying the preference of sexually naive females in estrus for cues of either control or infected males (Dass et al., 2011). We also compared the competitive ability of control and infected males to attract females. Uninfected females preferred parts of an arena containing urine marks deposited by infected males, compared with parts containing urine marks from control males (Dass et al., 2011). When females were allowed to choose between mating with either a control or an infected male, infected males consistently secured more intromissions and ejaculations. In the preceding experiment, a female mated in an arena housing one control and one infected male. Under non-competitive settings, i.e. where a female mated in an arena containing only one male, both control and infected males showed comparable reproductive performance. This distinction is important because it demonstrates that effects of infection were restricted to mate choice and not to the act of reproduction itself.

The role of *T. gondii* infection in rat mating success has been investigated previously (Berdoy et al., 1995). It was reported that infection did not alter mating success as defined by number of ejaculations and occurrences of mounting. Reproductive success of males in this arrangement is a product of both male–male competition and mate choice. One possibility is that females in this arena had less opportunity to control the timing and frequency of sexual interaction, for example by hiding in home-boxes that had an opening small enough to allow only females and not males. Several studies have established that intermittent cycles of solicitation and withdrawal by females is an essential feature of reproductive ritual in rats, and it facilitates successful pregnancy (e.g. Solomon and Keane, 2007). Another possibility is that male–male competition heavily contributed to the mating success in the semi-naturalistic setting, overriding the influence of female mate choice. This possibility will require further experimentation in the form of careful dissociation of both intra-sexual and inter-sexual behaviors. Pending that, it is difficult to ascertain whether parasitic manipulation of mate choice will result in a significant gain in reproductive fitness of infected males.

Mate choice often serves as a proxy of reproductive worth of a male because physiological conditions conducive to the production of sexual signals conflict with physiological or opportunistic demands of other life-history choices (Andersson, 1994; Andersson and Simmons, 2006). Sexual signals in many species are testosterone dependent. For example, in rats, females prefer males with greater testosterone (Taylor et al., 1982). Yet, testosterone is costly to produce and maintain (Alonso-Alvarez et al., 2009; Folstad and Karter, 1992; Wingfield et al., 1997). Firstly, the testosterone has immune-suppressive effects, leading to a trade-off on the part of males between higher testosterone and lowered capacity to fight infections. Secondly, testosterone facilitates behaviors that increase predation, leading to an opportunity cost.

In view of the centrality of testosterone to mate choice, we next asked whether *T. gondii* infection enhanced mate choice through enhanced testosterone. We demonstrated that *T. gondii* infection increased synthesis of testicular testosterone production in male rats (Lim et al., 2012). The majority of testosterone in male rats is

produced by Leydig cells present in the interstitial tissue of the testes. We compared the expression of steroidogenic enzymes in these cells using quantitative PCR. The infection enhanced the expression of steroidogenic enzymes, resulting in greater production of testosterone. What is interesting about these findings is that testosterone production was enhanced through a coordinated modulation of physiological checks and balances inherent to testicular steroidogenesis. Modulation of testosterone production has been well studied in the context of its decline in aging populations (Chen et al., 2009). The synthesis of testosterone is regulated by levels of luteinizing hormone secreted from the anterior pituitary gland. This hormone binds to the luteinizing hormone receptor (LHR) in Leydig cells of the testes, driving a second messenger system that successively recruits steroidogenic machinery (Azhar and Reaven, 2007; Payne, 2007). During aging, the number of LHRs in the Leydig cells drops, without significant changes in the amount of luteinizing hormone or the number of Leydig cells (Chen et al., 2009). The decrease in LHRs results in downregulation of cholesterol transport to the mitochondria and further biochemical modification of cholesterol, all necessary for testosterone production. The effects of *T. gondii* infection mirrored these physiological changes, albeit in the reverse direction. The infection enhanced expression of LHR without affecting luteinizing hormone. Furthermore, enhancement of LHR in infected animals resulted in upregulation of the same enzymes that are known to be diminished in aging. This points to a coordinated organizing principle that would be difficult to bring about by mere accidental effect or pathology, and thus represents a true physiological manipulation.

The effect of *T. gondii* infection on testosterone has previously been studied in mice and humans. In contrast to rats, *T. gondii* infection in mice reduces testosterone levels (Kaňková et al., 2011). We have observed that male mice infected with the parasite do not increase their sexual attractiveness to females, unlike male rats. While female mice respond to the infection with loss of innate fear, similar to male rats (Kannan et al., 2010; Vyas et al., 2007a), evidence for behavioral change in male mice has been rather mixed in our laboratory and published studies (e.g. Xiao et al., 2012). *Toxoplasma gondii* is more virulent in mice, often leading to sickness behavior and mortality (Hodková et al., 2007a; Hrdá et al., 2000), while infection in rats seldom results in sickness or death. It is likely that acute stress of infection in mice suppresses gonadal function (Sapolsky, 1985). It is currently not known why *T. gondii* is relatively more virulent in mice compared with rats. One possibility is that current laboratory strains of the parasite co-evolved with rats and not mice. In absence of any experimental evidence, this remains a speculation. In male human volunteers, *T. gondii* infection is associated with a statistically non-significant increase in salivary testosterone levels (Flegr et al., 2008). In parallel, female volunteers rate portraits of infected males as being more dominant and masculine compared with uninfected males (Hodková et al., 2007b) [see review by Flegr (Flegr, 2013) for a detailed discussion of toxoplasmosis in humans].

Apart from manipulating host mate choice, *T. gondii* also alters host response to predator odors (Berdoy et al., 2000; Vyas et al., 2007a; Vyas et al., 2007b; Webster and McConkey, 2010). Rats infected with *T. gondii* loose their natural aversion to cat odor and instead develop an attraction. This behavioral change is thought to be beneficial for the parasite as it can engage in sexual recombination only in the cat intestine. Moreover, passage through the cat provides the opportunity for oocysts to be widely disseminated in the environment, potentially infective to a wide range of other warm-blooded organisms. It is pertinent to ask here whether both behavioral changes – in mate choice and innate fear – are distinct or related. Two strands of evidence support the notion that parasitic changes in mate choice and predation are instead part of a single pattern built around testosterone-mediated interplay between mate choice and predation (Fig. 1) [see reviews by Webster et al. (Webster et al., 2013) and McConkey et al. (McConkey et al., 2013) for a detailed discussion of mechanisms].

Firstly, the greater testosterone levels necessary to augment mate choice can reduce the innate fear response through collateral actions in the brain. Testosterone is believed to reduce fear in a variety of species and experimental paradigms. Administration of exogenous testosterone reduces the fear response in humans and rodents (Aikey et al., 2002; Hermans et al., 2006; Kavaliers et al., 2001), while castration potentiates the fear response to predator odors in rodents (King et al., 2005). Testosterone also imparts attractiveness to males in birds, goats, rats and humans (Ardia et al., 2010; Bonisoli-Alquati et al., 2011; Bottoni et al., 1993; Longpre and Katz, 2011; Roney et al., 2006; Taylor et al., 1982). Parts of the limbic system that regulate fear contain generous amounts of receptors that bind to either testosterone or its aromatized metabolite, estrogen (Cooke, 2006; Kostarczyk, 1986; Swann and Fiber, 1997). These regions are sexually dimorphic, suggesting that testosterone plays an organizational role in sculpting them. Moreover, these regions retain structural and molecular plasticity in adulthood in response to testosterone. For example, castration produces epigenetic changes in arginine-vasopressin in the medial amygdala, a brain region important for regulation of social behavior (Auger et al., 2011). It is possible that parasite-induced enhancement of testosterone results in long-term plastic changes within the medial amygdala, resulting in a reduced fear response. Related to this, infected animals exhibit atypical activation of the posterodorsal part of the medial amygdala, which is responsible for sexual behaviors in normal situations and is highly responsive to testosterone (House et al., 2011). Furthermore, testosterone provides negative feedback to stress hormone secretion (Viau, 2002). In turn, stress hormones play a crucial facilitatory role

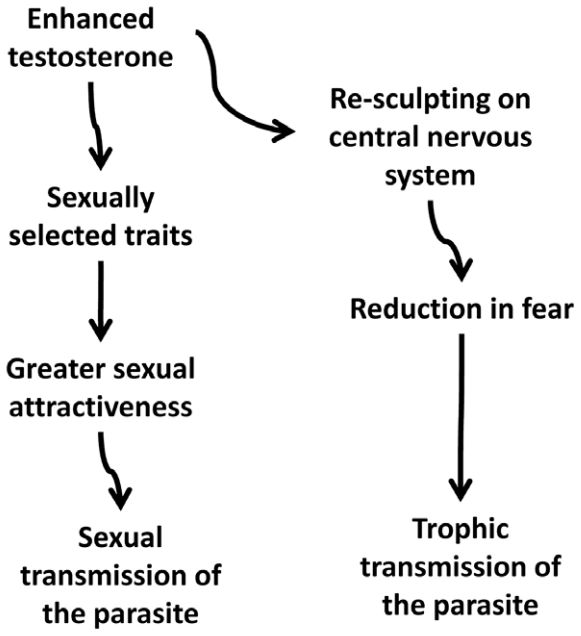

Fig.1. Possible role of testosterone in mediating host behavioral changes after infection with *Toxoplasma gondii*.

in generating and maintaining fear. For example, exogenous application of the stress hormone corticosterone results in plastic changes in the basolateral amygdala, resulting in excessive anxiety (Mitra and Sapolsky, 2008). Thus, testosterone could reduce fear response either directly through its action in the brain or indirectly through its suppressive effects on the stress response (Mitra et al., 2012). The main challenge for these possibilities is the fact that behavioral effects of *T. gondii* are rather specific (Lamberton et al., 2008; Vyas et al., 2007a; Vyas and Sapolsky, 2010). For example, although it affects innate fear, the infection does not change unconditioned anxiety or conditioned fear, although reduced anxiety has also been reported in rats (Gonzalez et al., 2007; Webster et al., 1994).

Secondly, parasite-augmented mate choice could enhance predation simply because testosterone enhances sexual signaling. Sexual signals are conspicuous and thus infected animals could be eaten more readily because they are easier to catch. This possibility has not been directly tested. An indirect observation supports this speculation. Rodents use odors to communicate. If infected animals enhance their sexual signaling, and if such sexual signaling can be intercepted as kairomones by other species, it can be argued that prey species will show greater aversion to odors from infected males. Mice are readily consumed by rats, and as a result mice exhibit a robust fear response to rat odors. In agreement with greater kairomonal activity, mice exhibit greater fear of urine from infected rats when compared with controls.

Thus, in short, *T. gondii* infection augments the attractiveness of infected males, plausibly through its effects on testosterone (Fig. 1). Collateral effects of testosterone could also explain loss of fear in infected rats (Fig. 1). It should be noted here that precise determination of reproductive fitness for rats or the parasite has not been carried out and remains a rather difficult task. The nocturnal habits and subterranean lifestyle of rats makes them a challenging species to study in field conditions. Moreover, it is difficult to determine whether the benefits of behavioral change outweigh the cost for the parasite or whether the cost of infection outweighs the benefits for the host. The host, the parasite, the predator and the prey reciprocally affect each other, leading to a complex cascade of effects. For example, the reproductive benefit to the infected rat due to enhanced attractiveness is countered by the cost of greater predation by cats and the cost of losing prey that can smell infected rats better than non-infected rats. In the absence of greater quantitative understanding of these trade-offs, it is very difficult to confirm the adaptive significance of the parasitic manipulation of mate choice. And it is even more difficult to determine whether parasite-augmented mate choice is frequent occurrence in nature or what kind of ecological situations favor such an association. A related issue is that of individual variation (Thomas et al., 2011). Not all rats respond similarly to cat odor or to *T. gondii* infection. For example, although the majority of rats show robust anxiety-like behaviors in response to cat cues, some individuals remain unaffected (Mitra et al., 2009). Similarly, not all infected animals develop an attraction to cat odors (Berdoy et al., 2000; Vyas et al., 2007a). Physiological and genetic substrates mediating this individual variation remain undetermined.

Parasite-augmented mate choice as an extended phenotype

Parasites change host behavior in a multitude of ways, and not all of these are behavioral manipulation by the parasite. Some of these changes are merely adaptive responses mounted by the host and some are side effects of host–parasite interactions. Poulin has proposed four criteria to recognize true manipulation (Poulin, 1995; Poulin, 2010): (1) complexity of effect; (2) purposiveness of design; (3) convergence in several lineages; and (4) increased fitness of the parasite due to manipulation (Poulin, 1995). Does parasite-augmented mate choice satisfy these criteria?

Complexity

A complex behavioral change requires an organizing principle and is less likely to be an accidental effect. Male sexual signaling is expensive to create and maintain. Sexually selected traits are limited by their physiological and opportunity costs. Infected males do not increase the strength of sexual display; in fact, they become less attractive. For example, female mice avoid males infected with *Eimeria vermiformis*, a close relative of *T. gondii*, and have an aversive physiological reaction to the smell of such males (Kavaliers and Colwell, 1992; Kavaliers and Colwell, 1995). *Toxoplasma gondii*-infected males overcome the cost of display, producing enough upregulation in their attractiveness to affect the behavior of another individual who herself is not infected, and these naive females change not only their mate choice but their actual mating decisions. This is a complex feat that arguably supports it being a co-evolutionary change (Poulin, 1995).

Purposiveness

A behavioral manipulation is ‘too well fitted’ to its purpose to arrive by chance. In other words, there needs to be conformity between the observed change and ‘*a priori* design specification that an engineer might use’ (Poulin, 1995). In this regard, making infected males more attractive to females *via* increased testosterone production is an ‘*a priori* design specification’ – an obvious solution to increase parasite transmission through sexual intercourse. Furthermore, physiological changes in infected males follow an organized logic similar to other biological situations where testosterone production is affected, albeit in the reverse direction.

Convergence

If similar effects arise in widely different lineages it is less likely that behavioral change is accidental. We are not aware of other examples where parasites augment attractiveness of infected males; the reverse is more often found. Related to this, increased circulating testosterone has also been observed in male rats infected with Seoul virus, a pathogen that is transmitted by wounding during aggressive encounters between males (Easterbrook et al., 2007). In addition, male midges infected by *Unionicola ypsilophora* have greater success at forming a mating pair (McLachlan, 1999), though the role of mate choice in this observation is not clear.

Fitness effects

A behavioral manipulation should lead to increased fitness of the parasite (Cézilly et al., 2010). We show that presence of behavioral manipulation leads to sexual opportunities in a competitive setting, which result in the transfer of semen containing parasites to females. These observations show that change in behavior correlates with parasite transmission. However, it must be noted that measurement of fitness is a difficult task. Moreover, fitness is often dependent on the environmental context in which experiments are conducted. The current state of research does not allow us to convincingly conclude that behavioral changes of infected rats indeed increase the fitness of the parasite.

Specificity

Although not one of Poulin’s criteria, the specificity of behavioral change is important. A generalized sickness behavior is

considerably less interesting than a specific behavioral change, for example increasing energy demand of the infected animal to the point of lethargy, leading to its predation. The case of *Toxoplasma* infection is different. Infected males do not have any deficit in reproductive behavior, they do not exhibit any sickness behavior, they do not lose weight after infection, and they sire a comparable number of offspring as non-infected males when mated with a female. Infection effects in this case are very specific to mate choice of uninfected females.

Conclusion

Several 'good gene' models assume that male sexual signaling is an 'honest' proxy of genetic legacy. I suggest here that in some cases parasites can manipulate this supposedly honest signaling by augmenting mate choice through enhanced testosterone. I further suggest that enhanced testosterone in the infected animals plays an important role in the reduction of innate fear observed in infected rats after *T. gondii* infection.

Funding

This research was funded by the Nanyang Assistant Professorship program of Nanyang Technological University, Singapore, and by the Ministry of Education, Singapore.

References

Able, D. J. (1996). The contagion indicator hypothesis for parasite-mediated sexual selection. *Proc. Natl. Acad. Sci. USA* **93**, 2229-2233.
Aikey, J. L., Nyby, J. G., Anmuth, D. M. and James, P. J. (2002). Testosterone rapidly reduces anxiety in male house mice (*Mus musculus*). *Horm. Behav.* **42**, 448-460.
Alonso-Alvarez, C., Pérez-Rodríguez, L., Garcia, J. T. and Viñuela, J. (2009). Testosterone-mediated trade-offs in the old age: a new approach to the immunocompetence handicap and carotenoid-based sexual signalling. *Proc. Biol. Sci.* **276**, 2093-2101.
Andersson, M. (1994). *Sexual Selection*. Princeton, NJ: Princeton University Press.
Andersson, M. and Simmons, L. W. (2006). Sexual selection and mate choice. *Trends Ecol. Evol.* **21**, 296-302.
Apfelbach, R., Blanchard, C. D., Blanchard, R. J., Hayes, R. A. and McGregor, I. S. (2005). The effects of predator odors in mammalian prey species: a review of field and laboratory studies. *Neurosci. Biobehav. Rev.* **29**, 1123-1144.
Arantes, T. P., Lopes, W. D., Ferreira, R. M., Pieroni, J. S., Pinto, V. M., Sakamoto, C. A. and Costa, A. J. (2009). *Toxoplasma gondii*: evidence for the transmission by semen in dogs. *Exp. Parasitol.* **123**, 190-194.
Ardia, D. R., Broughton, D. R. and Gleicher, M. J. (2010). Short-term exposure to testosterone propionate leads to rapid bill color and dominance changes in zebra finches. *Horm. Behav.* **58**, 526-532.
Auger, C. J., Coss, D., Auger, A. P. and Forbes-Lorman, R. M. (2011). Epigenetic control of vasopressin expression is maintained by steroid hormones in the adult male rat brain. *Proc. Natl. Acad. Sci. USA* **108**, 4242-4247.
Azhar, S. and Reaven, E. (2007). Regulation of Leydig cell cholesterol metabolism. In *Contemporary Endocrinology: The Leydig Cell in Health and Disease* (ed. A. H. Payne and M. P. Hardy), pp. 135-148. Totowa, NJ: Humana Press.
Baum, M. J. and Kelliher, K. R. (2009). Complementary roles of the main and accessory olfactory systems in mammalian mate recognition. *Annu. Rev. Physiol.* **71**, 141-160.
Berdoy, M., Webster, J. P. and Macdonald, D. W. (1995). Parasite-altered behaviour: is the effect of *Toxoplasma gondii* on *Rattus norvegicus* specific? *Parasitology* **111**, 403-409.
Berdoy, M., Webster, J. P. and Macdonald, D. W. (2000). Fatal attraction in rats infected with *Toxoplasma gondii*. *Proc. Biol. Sci.* **267**, 1591-1594.
Beynon, R. J., Hurst, J. L., Turton, M. J., Robertson, D. H. L., Armstrong, S. D., Cheetham, S. A., Simpson, D., MacNicoll, A. and Humphries, R. E. (2008). Urinary lipocalins in Rodenta: is there a generic model? In *Chemical Signals in Vertebrates*, Vol. 11 (ed. J. Hurst, R. J. Beynon, S. C. Roberts and T. Wyatt), pp. 37-50. New York, NY: Springer.
Bonisoli-Alquati, A., Matteo, A., Ambrosini, R., Rubolini, D., Romano, M., Caprioli, M., Dessi-Fulgheri, F., Baratti, M. and Saino, N. (2011). Effects of egg testosterone on female mate choice and male sexual behavior in the pheasant. *Horm. Behav.* **59**, 75-82.
Bottoni, L., Massa, R., Lea, R. W. and Sharp, P. J. (1993). Mate choice and reproductive success in the red-legged partridge (*Alectoris rufa*). *Horm. Behav.* **27**, 308-317.
Cézilly, F., Thomas, F., Médoc, V. and Perrot-Minnot, M. J. (2010). Host-manipulation by parasites with complex life cycles: adaptive or not? *Trends Parasitol.* **26**, 311-317.
Chen, H., Ge, R. S. and Zirkin, B. R. (2009). Leydig cells: from stem cells to aging. *Mol. Cell. Endocrinol.* **306**, 9-16.
Cooke, B. M. (2006). Steroid-dependent plasticity in the medial amygdala. *Neuroscience* **138**, 997-1005.
Dass, S. A., Vasudevan, A., Dutta, D., Soh, L. J., Sapolsky, R. M. and Vyas, A. (2011). Protozoan parasite *Toxoplasma gondii* manipulates mate choice in rats by enhancing attractiveness of males. *PLoS ONE* **6**, e27229.
Dawkins, R. (1982). *The Extended Phenotype: The Long Reach of the Gene*. Oxford: Oxford University Press.
de Moraes, E. P., Batista, A. M., Faria, E. B., Freire, R. L., Freitas, A. C., Silva, M. A., Braga, V. A. and Mota, R. A. (2010). Experimental infection by *Toxoplasma gondii* using contaminated semen containing different doses of tachyzoites in sheep. *Vet. Parasitol.* **170**, 318-322.
Easterbrook, J. D., Kaplan, J. B., Glass, G. E., Pletnikov, M. V. and Klein, S. L. (2007). Elevated testosterone and reduced 5-HIAA concentrations are associated with wounding and hantavirus infection in male Norway rats. *Horm. Behav.* **52**, 474-481.
Ehman, K. D. and Scott, M. E. (2002). Female mice mate preferentially with non-parasitized males. *Parasitology* **125**, 461-466.
Endler, J. A. and Lyles, A. M. (1989). Bright ideas about parasites. *Trends Ecol. Evol.* **4**, 246-248.
Fisher, R. A. (1915). The evolution of sexual preference. *Eugen. Rev.* **7**, 184-192.
Flegr, J. (2013). Influence of latent *Toxoplasma* infection on human personality, physiology and morphology: pros and cons of the *Toxoplasma*–human model in studying the manipulation hypothesis. *J. Exp. Biol.* **216**, 127-133.
Flegr, J., Lindová, J. and Kodym, P. (2008). Sex-dependent toxoplasmosis-associated differences in testosterone concentration in humans. *Parasitology* **135**, 427-431.
Folstad, I. and Karter, A. K. (1992). Parasites, bright males and the immunocompetence handicap. *Am. Nat.* **139**, 603-622.
Getty, T. (2002). Signaling health versus parasites. *Am. Nat.* **159**, 363-371.
Gonzalez, L. E., Rojnik, B., Urrea, F., Urdaneta, H., Petrosino, P., Colasante, C., Pino, S. and Hernandez, L. (2007). *Toxoplasma gondii* infection lower anxiety as measured in the plus-maze and social interaction tests in rats: a behavioral analysis. *Behav. Brain Res.* **177**, 70-79.
Hall, D. W., Kirkpatrick, M. and West, B. (2000). Runaway sexual selection when female preferences are directly selected. *Evolution* **54**, 1862-1869.
Hamilton, W. D. and Zuk, M. (1982). Heritable true fitness and bright birds: a role for parasites? *Science* **218**, 384-387.
Haynes, K. F. and Yeargan, K. V. (1999). Exploitation of intraspecific communication systems: illicit signalers and receivers. *Ann. Entomol. Soc. Am.* **92**, 960-970.
Hermans, E. J., Putman, P., Baas, J. M., Koppeschaar, H. P. and van Honk, J. (2006). A single administration of testosterone reduces fear-potentiated startle in humans. *Biol. Psychiatry* **59**, 872-874.
Hodková, H., Kodym, P. and Flegr, J. (2007a). Poorer results of mice with latent toxoplasmosis in learning tests: impaired learning processes or the novelty discrimination mechanism? *Parasitology* **134**, 1329-1337.
Hodková, H., Kolbeková, P., Skallová, A., Lindová, J. and Flegr, J. (2007b). Higher perceived dominance in *Toxoplasma* infected men – a new evidence for role of increased level of testosterone in toxoplasmosis-associated changes in human behavior. *Neuroendocrinol. Lett.* **28**, 110-114.
House, P. K., Vyas, A. and Sapolsky, R. (2011). Predator cat odors activate sexual arousal pathways in brains of *Toxoplasma gondii* infected rats. *PLoS ONE* **6**, e23277.
Hrdá, S., Votýpka, J., Kodym, P. and Flegr, J. (2000). Transient nature of *Toxoplasma gondii*-induced behavioral changes in mice. *J. Parasitol.* **86**, 657-663.
John, J. L. (1997). The Hamilton-Zuk theory and initial test: an examination of some parasitological criticisms. *Int. J. Parasitol.* **27**, 1269-1288.
Jones, A. G. and Ratterman, N. L. (2009). Mate choice and sexual selection: what have we learned since Darwin? *Proc. Natl. Acad. Sci. USA* **106 Suppl. 1**, 10001-10008.
Kaňková, S., Kodym, P. and Flegr, J. (2011). Direct evidence of *Toxoplasma*-induced changes in serum testosterone in mice. *Exp. Parasitol.* **128**, 181-183.
Kannan, G., Moldovan, K., Xiao, J. C., Yolken, R. H., Jones-Brando, L. and Pletnikov, M. V. (2010). *Toxoplasma gondii* strain-dependent effects on mouse behaviour. *Folia Parasitol.* **57**, 151-155.
Kavaliers, M. and Choleris, E. (2011). Sociality, pathogen avoidance, and the neuropeptides oxytocin and arginine vasopressin. *Psychol. Sci.* **22**, 1367-1374.
Kavaliers, M. and Colwell, D. D. (1992). Exposure to the scent of male mice infected with the protozoan parasite, *Eimeria vermiformis*, induces opioid- and nonopioid-mediated analgesia in female mice. *Physiol. Behav.* **52**, 373-377.
Kavaliers, M. and Colwell, D. D. (1995). Discrimination by female mice between the odours of parasitized and non-parasitized males. *Proc. Biol. Sci.* **261**, 31-35.
Kavaliers, M., Colwell, D. D. and Choleris, E. (1998a). Analgesic responses of male mice exposed to the odors of parasitized females: effects of male sexual experience and infection status. *Behav. Neurosci.* **112**, 1001-1011.
Kavaliers, M., Colwell, D. D. and Choleris, E. (1998b). Parasitized female mice display reduced aversive responses to the odours of infected males. *Proc. Biol. Sci.* **265**, 1111-1118.
Kavaliers, M., Choleris, E. and Colwell, D. D. (2001). Brief exposure to female odors "emboldens" male mice by reducing predator-induced behavioral and hormonal responses. *Horm. Behav.* **40**, 497-509.
Kavaliers, M., Colwell, D. D., Choleris, E., Agmo, A., Muglia, L. J., Ogawa, S. and Pfaff, D. W. (2003). Impaired discrimination of and aversion to parasitized male odors by female oxytocin knockout mice. *Genes Brain Behav.* **2**, 220-230.
Kavaliers, M., Choleris, E. and Pfaff, D. W. (2005a). Genes, odours and the recognition of parasitized individuals by rodents. *Trends Parasitol.* **21**, 423-429.
Kavaliers, M., Choleris, E. and Pfaff, D. W. (2005b). Recognition and avoidance of the odors of parasitized conspecifics and predators: differential genomic correlates. *Neurosci. Biobehav. Rev.* **29**, 1347-1359.
Kavaliers, M., Choleris, E., Agmo, A., Braun, W. J., Colwell, D. D., Muglia, L. J., Ogawa, S. and Pfaff, D. W. (2006). Inadvertent social information and the avoidance of parasitized male mice: a role for oxytocin. *Proc. Natl. Acad. Sci. USA* **103**, 4293-4298.

King, J. A., De Oliveira, W. L. and Patel, N. (2005). Deficits in testosterone facilitate enhanced fear response. *Psychoneuroendocrinology* **30**, 333-340.
Klein, S. L. and Nelson, R. J. (1999). Activation of the immune-endocrine system with lipopolysaccharide reduces affiliative behaviors in voles. *Behav. Neurosci.* **113**, 1042-1048.
Kostarczyk, E. M. (1986). The amygdala and male reproductive functions: I. Anatomical and endocrine bases. *Neurosci. Biobehav. Rev.* **10**, 67-77.
Lafferty, K. D. (1999). The evolution of trophic transmission. *Parasitol. Today* **15**, 111-115.
Lamberton, P. H., Donnelly, C. A. and Webster, J. P. (2008). Specificity of the *Toxoplasma gondii*-altered behaviour to definitive versus non-definitive host predation risk. *Parasitology* **135**, 1143-1150.
Lim, A., Kumar, V., Hari Dass, S. A. and Vyas, A. (2012). *Toxoplasma gondii* infection enhances testicular steriodogeneis in rat. *Mol. Ecol.* doi: 10.1111/mec.12042.
Liu, S. G., Qin, C., Yao, Z. J. and Wang, D. (2006). Study on the transmission of *Toxoplasma gondii* by semen in rabbits. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* **24**, 166-170.
Longpre, K. M. and Katz, L. S. (2011). Estrous female goats use testosterone-dependent cues to assess mates. *Horm. Behav.* **59**, 98-104.
McConkey, G. A., Martin, H. L., Bristow, G. C. and Webster, J. P. (2013). *Toxoplasma gondii* infection and behaviour – location, location, location? *J. Exp. Biol.* **216**, 113-119.
McLachlan, A. (1999). Parasites promote mating success: the case of a midge and a mite. *Anim. Behav.* **57**, 1199-1205.
Mitra, R. and Sapolsky, R. M. (2008). Acute corticosterone treatment is sufficient to induce anxiety and amygdaloid dendritic hypertrophy. *Proc. Natl. Acad. Sci. USA* **105**, 5573-5578.
Mitra, R., Adamec, R. and Sapolsky, R. (2009). Resilience against predator stress and dendritic morphology of amygdala neurons. *Behav. Brain Res.* **205**, 535-543.
Mitra, R., Sapolsky, R. M. and Vyas, A. (2012). *Toxoplasma gondii* infection induces dendritic retraction in basolateral amygdala accompanied by reduced corticosterone secretion. *Dis. Model Mech.* doi:10.1242/dmm.009928.
Møller, A. P. and Jennions, M. D. (2001). How important are direct fitness benefits of sexual selection? *Naturwissenschaften* **88**, 401-415.
Moore, J. (2000). *Parasites and the Behavior of Animals.* Oxford: Oxford University Press.
Payne, A. H. (2007). Steroidogenic enzymes in Leydig cells. In *Contemporary Endocrinology: The Leydig Cell in Health and Disease* (ed. A. H. Payne and M. P. Hardy), pp. 157-171. Totowa, NJ: Humana Press.
Poulin, R. (1995). "Adaptive" changes in the behaviour of parasitized animals: a critical review. *Int. J. Parasitol.* **25**, 1371-1383.
Poulin, R. (2010). Parasite manipulation of host behavior: an update and frequently asked questions. *Adv. Study Behav.* **41**, 151-186.
Read, A. F. (1988). Sexual selection and the role of parasites. *Trends Ecol. Evol.* **3**, 97-102.
Rivière, S., Challet, L., Fluegge, D., Spehr, M. and Rodriguez, I. (2009). Formyl peptide receptor-like proteins are a novel family of vomeronasal chemosensors. *Nature* **459**, 574-577.
Roney, J. R., Hanson, K. N., Durante, K. M. and Maestripieri, D. (2006). Reading men's faces: women's mate attractiveness judgments track men's testosterone and interest in infants. *Proc. Biol. Sci.* **273**, 2169-2175.
Roy, A. K., Chatterjee, B., Rao, K. V., Murty, C. V., Sarkar, F. H. and Majumdar, D. (1987). Androgenic regulation of hepatic gene expression. *J. Steroid Biochem.* **27**, 1129-1134.
Sapolsky, R. M. (1985). Stress-induced suppression of testicular function in the wild baboon: role of glucocorticoids. *Endocrinology* **116**, 2273-2278.
Smith, J. M. (1991). Theories of sexual selection. *Trends Ecol. Evol.* **6**, 146-151.
Soares, V. C., Gubits, R. M., Feigelson, P. and Costantini, F. (1987). Tissue-specific and hormonally regulated expression of a rat alpha 2u globulin gene in transgenic mice. *Mol. Cell. Biol.* **7**, 3749-3758.
Solomon, N. G. and Keane, B. (2007). Reproductive strategies in female rodents. In *Rodent Societies: an Ecological and Evolutionary Perspective* (ed. J. O. Wolff and P. W. Sherman), pp. 42-56. Chicago, IL: University of Chicago Press.
Swann, J. and Fiber, J. M. (1997). Sex differences in function of a pheromonally stimulated pathway: role of steroids and the main olfactory system. *Brain Res. Bull.* **44**, 409-413.
Taylor, G. T., Haller, J. and Regan, D. (1982). Female rats prefer an area vacated by a high testosterone male. *Physiol. Behav.* **28**, 953-958.
Thomas, F., Brodeur, J., Maure, F., Franceschi, N., Blanchet, S. and Rigaud, T. (2011). Intraspecific variability in host manipulation by parasites. *Infect. Genet. Evol.* **11**, 262-269.
Tirindelli, R., Dibattista, M., Pifferi, S. and Menini, A. (2009). From pheromones to behavior. *Physiol. Rev.* **89**, 921-956.
Viau, V. (2002). Functional cross-talk between the hypothalamic-pituitary-gonadal and -adrenal axes. *J. Neuroendocrinol.* **14**, 506-513.
Vyas, A. and Sapolsky, R. (2010). Manipulation of host behaviour by *Toxoplasma gondii*: what is the minimum a proposed proximate mechanism should explain? *Folia Parasitol.* **57**, 88-94.
Vyas, A., Kim, S. K., Giacomini, N., Boothroyd, J. C. and Sapolsky, R. M. (2007a). Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. *Proc. Natl. Acad. Sci. USA* **104**, 6442-6447.
Vyas, A., Kim, S. K. and Sapolsky, R. M. (2007b). The effects of toxoplasma infection on rodent behavior are dependent on dose of the stimulus. *Neuroscience* **148**, 342-348.
Webster, J. P. and McConkey, G. A. (2010). *Toxoplasma gondii*-altered host behaviour: clues as to mechanism of action. *Folia Parasitol.* **57**, 95-104.
Webster, J. P., Brunton, C. F. and MacDonald, D. W. (1994). Effect of *Toxoplasma gondii* upon neophobic behaviour in wild brown rats, *Rattus norvegicus*. *Parasitology* **109**, 37-43.
Webster, J. P., Kaushik, M., Bristow, G. C. and McConkey, G. A. (2013). *Toxoplasma gondii* infection, from predation to schizophrenia: can animal behaviour help us understand human behaviour? *J. Exp. Biol.* **216**, 99-112.
Willis, C. and Poulin, R. (2000). Preference of female rats for the odours of non-parasitised males: the smell of good genes? *Folia Parasitol.* **47**, 6-10.
Wingfield, J. C., Jacobs, J. and Hillgarth, N. (1997). Ecological constraints and the evolution of hormone-behavior interrelationships. *Ann. N. Y. Acad. Sci.* **807**, 22-41.
Xiao, J., Kannan, G., Jones-Brando, L., Brannock, C., Krasnova, I. N., Cadet, J. L., Pletnikov, M. and Yolken, R. H. (2012). Sex-specific changes in gene expression and behavior induced by chronic *Toxoplasma* infection in mice. *Neuroscience* **206**, 39-48.
Zala, S. M., Chan, B. K., Bilbo, S. D., Potts, W. K., Nelson, R. J. and Penn, D. J. (2008). Genetic resistance to infection influences a male's sexual attractiveness and modulation of testosterone. *Brain Behav. Immun.* **22**, 381-387.
Zuk, M. and Kolluru, G. R. (1998). Exploitation of sexual signals by predators and parasitoids. *Q. Rev. Biol.* **73**, 415-438.
Zuk, M., Rotenberry, J. T. and Tinghitella, R. M. (2006). Silent night: adaptive disappearance of a sexual signal in a parasitized population of field crickets. *Biol. Lett.* **2**, 521-524.