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Host Behavioral Manipulation by *Toxoplasma gondii*: Integrating Neurobiological and Evolutionary Mechanisms

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Abstract

Toxoplasma gondii is a parasite with an alternating life cycle between sexual reproduction in felids (definitive host) and asexual replication in any other mammal or bird (intermediate host). This parasite is thought to manipulate its intermediate hosts by increasing the attractiveness of males, decreasing the aversion to cat odor and adjusting the secondary sex ratio (SSR). The evidence for these behavioral manipulations is strong in rodents, but only the SSR manipulation is well-studied and confirmed in humans. While increased testosterone levels are proposed as the underlying mechanism for all three phenomena, this is only based on clear evidence for the increased attractiveness of males and not for the other behavioral modifications. Other proposed mechanisms include the selective tropism and dopamine production of neurological cysts and neuroinflammation for the decreased aversion to cat odor, and immunosuppression and the Trivers-Willard hypothesis for the manipulated SSR. One outstanding question is the effect of these manipulations on the parasite's evolutionary fitness in rodents and human beings. Strong indirect evidence indicates that, in rodents, the increased attractiveness of infected males will enhance the sexual transmission, and the decreased aversion to cat odor will enhance the trophic transmission of the parasite, suggesting that these manipulations are adaptive for *T. gondii*. Moreover, the manipulation of the SSR could facilitate long-range and trophic transmission in rodents, although the evidence for this is less strong. However, only the increased attractiveness of infected males could be adaptive for this parasite in modern humans, since the human host breaks the life cycle of this parasite. This suggests divergent effects on *Toxoplasma* evolutionary fitness of behavioral modifications in different hosts.

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Introduction

Host-parasite interactions are often considered an evolutionary arms race (1). Both the parasite and host develop adaptations and counter-adaptations against each other, resulting in antagonistic coevolution (1). The Red Queen hypothesis states this evolution potentially occurs rapidly with little obvious effect on fitness (2). With hosts evolving increasingly stronger defense mechanisms to avoid parasites and/or their pathogenicity, parasites have to evolve new ways of infecting and exploiting hosts (3).

Manipulation of host behavior by parasites is quite a common phenomenon in nature. Hereby, the behavior of the host becomes an extended phenotype of the parasite's genes (4). These alterations in host behavior are often considered adaptive for the parasite, since they can increase transmission to new hosts, enhance parasite survival or ensure parasite release in an appropriate location (3). A classic example of such a behavioral manipulation comes from crickets that commit suicide by jumping into bodies of water following infection by hairworms, allowing the adult worm to complete the free-living, aquatic, reproductive part of its life cycle (5). Another example is the manipulation of potato aphids by the larvae of parasitic wasp, which differs depending on whether the larvae exhibit diapause (delayed development) or not (6). Non-diapausing larvae make their host climb to the upper surface of the leaves before mummification (during which time the larvae devour the host), while diapausing larvae make their host seek concealment before they mummify to protect themselves from hyperparasitoids during the diapause. Moreover, many trophically transmitted parasites alter the behavior of their intermediate hosts to enhance transmission through predation by their definitive hosts (7,8,9).

Toxoplasma gondii is such a trophically transmitted parasite. After the first observation of altered behavior in infected rodents in the late 1970s, it was hypothesized that this protozoan could modify the behavior of these intermediate hosts (10). However, *T. gondii* does not only infect rodents. Approximately one third of the human population worldwide is also infected by this parasite (11). The possibility that human behavior could also be influenced by the infection has generated continued interest for *T. gondii*, with dozens of studies linking it to a wide range of behavioral modifications in rodents and humans. Even diseases such as schizophrenia, depression and obsessive-compulsive disorder have already been associated with its presence (11).

One outstanding question in this field is the effects of behavior modification on parasite fitness. In this thesis, I will exclusively examine the three proposed host manipulations by *T. gondii* that have the potential to be adaptive for the parasite. These include increased attractiveness of males (12), a decreased aversion to cat odor (13) and adjustment of the secondary sex ratio (14). I will discuss the evidence and proposed evolutionary and neurobiological mechanisms for these manipulations in rodents and humans, and for each I will address the question of whether the manipulation is adaptive for *T. gondii* or not. To be able to understand the mechanisms and adaptive potential of these manipulations, it is essential to first explain the complex life cycle of this parasite in more detail.

Life Cycle of *Toxoplasma gondii*

The life cycle of *T. gondii* alternates between sexual reproduction in the definitive host and asexual replication in the intermediate host (figure 1). While the asexual component can probably occur in almost any mammal or bird (15), the sexual component is restricted to felids (domestic and wild cats, 16), since they are the only mammals with a systemic excess of linoleic acid (17), which is essential for the sexual reproduction of *T. gondii* (18). This excess is due to the lack of delta-6-desaturase activity in their intestines preventing linoleic acid metabolism (17). The *Toxoplasma* life cycle contains three cellular stages: rapidly-proliferating tachyzoites that invade tissues, more slowly dividing bradyzoites occurring within tissue cysts, and infectious sporozoites that persist as oocysts in the environment.

The asexual cycle begins when an intermediate host gets infected by *T. gondii* after ingestion of oocysts (mainly by herbivores) or tissue cysts (by carnivores). The wall of the cyst or oocyst are destroyed by gastric enzymes, freeing bradyzoites or sporozoites into the intestinal lumen. Here they enter the intestinal epithelium and differentiate into tachyzoites, which rapidly proliferate through asexual multiple fission (schizogony) and spread throughout the body. Pressure of the host's immune system causes them to differentiate into bradyzoites which cluster in host tissues forming cysts. Since the brain, eyes and testes are immune-privileged, tissue cysts will mainly develop in these sites (19).

The sexual cycle begins when a felid consumes an infected intermediate host. Bradyzoites from the cysts of the prey animal infect the feline intestinal epithelium just as they do in the intermediate host, but in the presence of high levels of linoleic acid, schizogony is followed by the formation of male and female gametes (gametogony), which form oocysts after fertilization. An infected cat starts shedding these oocysts through its feces a few days after ingestion of tissue cysts and sheds more than 100 million over the following 20 days. Over the course of a few days in the external environment, a process called sporogony, involving meiotic reduction, results in an oocyst containing two sporocysts each with four sporozoites. The oocysts are now mature and infective for new hosts. Oocysts are very resistant and remain infective for a long time in water, soil and on plants, but also in and on insects. (20-22)

Tachyzoites often do not survive ingestion (22), but their invasive character enables non-trophic forms of transmission. Vertical transmission of tachyzoites occurs by transplacental transport during pregnancy (21) and horizontal transmission via the semen of infected males (12,28-32). Since tachyzoites are only present in recently infected hosts, these forms of transmission are generally only possible in early stages of infection (23-27). Having described the fundamentals of the life cycle, I will now proceed to review the three behavioral modifications that are potentially adaptive for *T. gondii*.

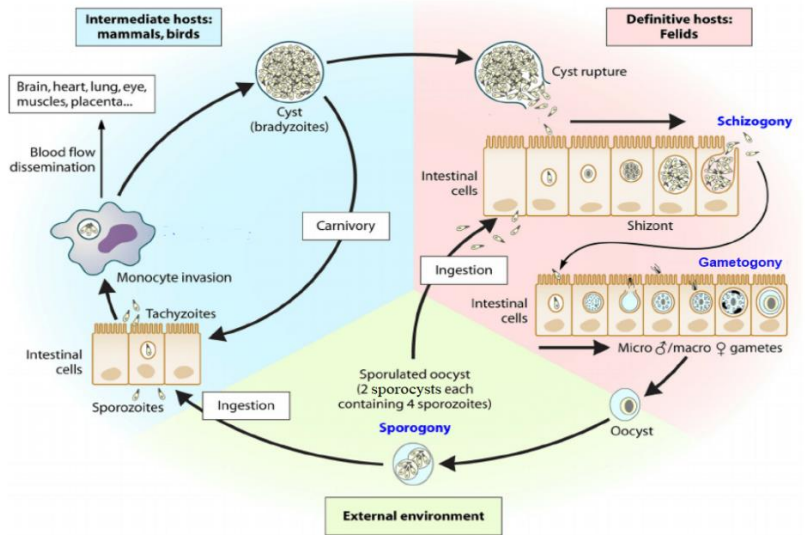

Figure 1: The life cycle of *T. gondii* (21)

Increased Attractiveness of Infected Males

It is often proposed that *T. gondii* infection can increase the attractiveness of males (expressed here by the female preference score, the ratio between time spent in infected and control bisects). A study in rats demonstrated that uninfected females had a significant preference (preference score 1.54) for urine marks deposited by *Toxoplasma*-infected males in comparison with control marks in a two-choice test with the males present at the end of the bisect (12). When repeated with males absent, to exclude a role for vocalizations and other interactions, similar results were obtained (preference score 1.30). Uninfected females also exhibited this preference when they were allowed to choose to mate with either an infected or an uninfected male: infected males accomplished significantly more intromissions. This was not due to differences in reproductive performance between the males, since they showed a similar number of mounts and intromissions when mating individually with a female. Female preference for the urine of infected males was confirmed by a similar experiment in another study, where the preference score (2.12) was even higher (33). However, an earlier study in rats reported no difference in mating success between infected and uninfected males (34). This could be attributed to the experimental design, which allowed male-male competition and did not provide appropriate hiding places for female withdrawal (35), an essential part of their courtship behavior (36). A study in Prague suggested that *T. gondii* could also enhance male attractiveness in humans, since infected men were perceived as more dominant by women based on portrait pictures (37), and the social dominance of men is believed to be a trait found attractive by heterosexual women according to psychological research (38).

Proposed Mechanism

Enhanced testosterone production in *Toxoplasma*-infected males is the proposed mechanism for their increased attractiveness. A study in male rats showed that testicular testosterone concentrations more than doubled after infection (39). Testicular Leydig cells produce most of the testosterone in males (39), involving multiple metabolic steps ultimately driven by luteinizing hormone (figure 2). The expression of the luteinizing hormone receptor (LHR) was significantly enhanced (10.5-fold) in the infected male rats compared to controls, resulting in increased expression of several steroidogenic enzymes: the mRNA expression levels of StAR, $P450_{scc}$, 17β-HSD and $P450_{C17a}$ were increased respectively by 2.65-, 1.65-, 8.6- and 15.9-fold. However, the expression of aromatase and 3β-HSD did not differ, which is particularly interesting since these enzymes specifically generate 'female' hormones.

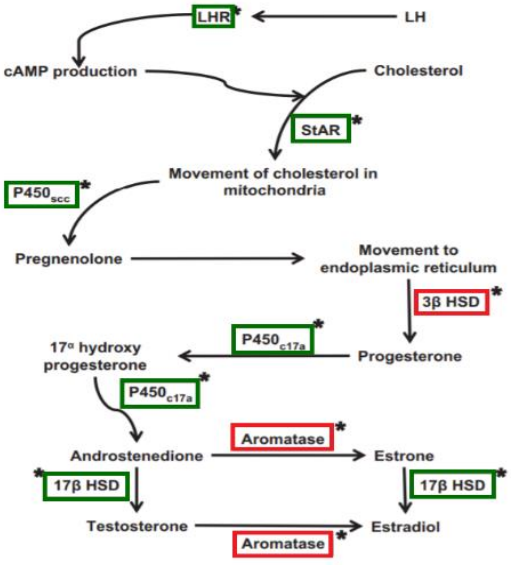

Figure 2: The metabolic pathway of testosterone production. The expression of proteins marked by green rectangles was enhanced in infected male rats, while the expression of proteins marked by red rectangles did not differ between infected and uninfected animals (39)

One way by which testosterone can affect male attractiveness is by enhancing the production of α2u-globulin, which is a major urinary protein of male rats. A study in rats demonstrated that both the hepatic production and urinary excretion of these globulins was significantly higher in *Toxoplasma*-infected males compared to controls (33). Moreover, uninfected females showed a strong preference for purified α2u-globulins from the urine of infected males. When given a choice between high and low doses of this protein, uninfected females showed a preference for the higher concentration. These observations suggest that α2u-globulins are sufficient to enhance the attractiveness of infected male rats in a dose-dependent manner. The increased production of α2u-globulins in these males was probably due to increased testosterone production (39), since testosterone is required for the α2u-globulin synthesis (33,40).

Increased testosterone levels were also reported in infected men by several human studies (41-44), although one Iranian study showed decreased testosterone levels (45). However, there is no human genetic homolog of rodent α2u-globulin, owing either to gene family expansion in the rodent lineage or gene loss in the primate lineage (46). Therefore, if *T. gondii* infection does actually increase the attractiveness of men, the effect must arise by another mechanism. This is not terribly surprising, since aroma appears to play a more important role in rodent social discrimination, while vision is more important in human beings (47); possible mechanisms operating in humans are therefore visual, for example by making men's faces look more masculine (48).

Species differences in the mechanisms by which male attractiveness may be modified by *T. gondii* infection are not restricted to humans versus rodents. Specifically, no preference of uninfected females for infected compared to uninfected males was observed in mice (35,49). Consistent with the idea that testosterone is responsible for this female preference, and in contrast to what is seen in rats, infected male mice appeared to have decreased testosterone levels (50). Since testosterone is known to have immunosuppressive effects (51) and *T. gondii* is more virulent in mice than in rats leading to a much higher frequency of sickness behavior and mortality (50), reduced testosterone in infected mice may be an adaptation to compensate for the immunosuppression caused by *T. gondii* infection (50). This is supported by the observation that testosterone levels were also decreased in female mice (50). Nothing is currently known about attractiveness of infected males in other species such as birds.

Adaptive Potential

Female rodents typically avoid males that are infected by parasites (52-54). This response has probably evolved because it selects for males with a better heritable parasite resistance (55) and/or prevents sexual transmission of parasites to the female (56). This can strongly inhibit horizontal transmission of sexually transmitted parasites and natural selection operating on *T. gondii* may therefore select for a manipulation of host reproductive behavior to overcome this. Male sexual advertisement is thought to be an honest signal of the quality of their immune system (57), since it often requires testosterone which produces a handicap in the ability to fight parasites by suppressing immune function (51,57). Sexual advertisement of *Toxoplasma*-infected males is not honest, since *T. gondii* manipulates it by increasing the level of testosterone. The resulting female preference for infected males likely increases horizontal sexual transmission of *T. gondii* (35). Moreover, if this sexual transmission occurs shortly before conceiving, it can also enhance the vertical transmission from mother to offspring via the placenta (25-27).

Fatal Attraction Phenomenon

Another proposed behavioral manipulation by *T. gondii* is the so-called 'fatal attraction phenomenon'. Although *Toxoplasma*-free rodents have an innate aversion to cat odors (58), a study in the year 2000 was the first to report that rats infected by *T. gondii* could lose this fear (13). Infected rats were significantly less averse towards cat urine than controls and showed overall no avoidance of it, while uninfected rats demonstrated a normal aversion. In the following years, numerous studies in mice and rats obtained similar results (39,59-69), despite small heterogeneities as summarized in table 1. Some studies even reported not just a loss of aversion, but an attraction of infected rodents towards cat odor (13,59,64). Moreover, *T. gondii* appeared to specifically abolish the aversion to the odors of felids, since the behavioral response of infected animals did not change to odors of non-predatory animals such as rabbits (13,59,60) or to the odors of predators that are not important for the sexual part of its life cycle such as dogs (61) and mink (62,65). However, one study did not report this specificity (64). A study in rats showed that the decrease in aversion to feline odors of infected animals was dependent on the dose of the stimulus (63), with roughly a maximal decrease for intermediate doses of cat odor.

Effect on cat odor aversion	**Behavioral test**	**Host**	***T. gondii* Strain**	**Study**
Urine aversion decreased	Four-choice test between cat urine, water, own smell and rabbit urine	Lister-Hooded rats	Beverly	(13)
Urine aversion decreased in both sexes, fur aversion decreased only in females	Two-choice test between cat and rabbit urine or worn and unworn cat collar	Male Long-Evans rats, female BALB/c mice	PRU	(59)
Urine aversion decreased	Four-choice test between cat urine, water, own smell and rabbit urine	Lister-Hooded rats	ME49	(60)
Urine aversion decreased 2 months post-infection, not 7 months post-infection	Two-choice test between cat and dog urine	Female BALB/c mice	PRU, ME49	(61)
Urine aversion decreased	Two-choice test between cat and mink urine	Lister-Hooded rats	ME49	(62)
Urine and fur aversion dose-dependently decreased	Two-choice test between different doses of cat urine and neutral zone or cat and unscented towel	Male Long-Evans rats	PRU	(63)
Urine aversion decreased	Two-choice test between cat urine and own odor	Male B6CBAF1/J mice	ME49	(64)
Urine aversion decreased only in females	Two-choice test between cat and mink urine	Male and female BALB/c mice	PRU	(65)
Urine aversion decreased	Two-choice test between different types of cat urine	Male and female Lister-Hooded rats	PRU	(66)
Urine aversion decreased	Two-choice test between cat and rabbit urine	Male Wistar rats	PRU	(67)
Urine aversion decreased	Two-choice test between cat and rabbit urine	Male Wistar rats	PRU	(39)
Urine aversion decrease dependent on estrus cycle	Two-choice test between cat and rabbit urine	Female Long-Evans rats	PRU	(68)
Urine aversion decreased	Open field test with cat urine	Male BALB/c mice	CEP, RHΔrop5	(69)

Table 1: Previous studies on the effect of *T. gondii* infection on rodent aversion towards cat urine

A study in Prague suggested that this phenomenon may also be present in humans, although the effect of the infection on the experienced pleasantness of cat urine was strongly gender-dependent: infected males rated the urine significantly more pleasant than uninfected males, while infected females rated it less pleasant than uninfected females (70).

Furthermore, several studies in rodents and humans reported changes in more general behavioral components like risky behavior, learning and memory, anxiety and activity level after *T. gondii* infection (71,72). However, there are many inconsistencies between these studies and their relevance to feline predation is unclear. Therefore, I will not discuss these behavioral changes in further detail here.

Proposed Mechanisms

Testosterone and Epigenetics Hypothesis

Increased testosterone production in *Toxoplasma*-infected males, as described above, could also be the mechanism underlying the fatal attraction phenomenon. Testosterone is known to bind to its receptors in the medial amygdala (73), containing neurons that express the neurotransmitter arginine vasopressin, which mediates reproductive behaviors (74). A study in male rats showed a decreased methylation of the arginine vasopressin promoter in the dorsal medial amygdala of infected animals compared to controls, leading to locally enhanced expression of this neurotransmitter (67). Moreover, induced hypermethylation abolished the increased attraction to cat urine in the infected rats, while it did not influence the aversion of uninfected animals, demonstrating the necessity of hypomethylation for this phenomenon. Its sufficiency was demonstrated by a reduced aversion to cat odor in uninfected rats after administration of a DNA methyltransferase inhibitor in their dorsal medial amygdala. Furthermore, significantly more arginine vasopressin neurons were activated in this brain region of infected rats compared to controls during exposure to cat urine. Without exposure their activity was not influenced by the infection, demonstrating that not the infection status itself, but the combination of the infection with cat odor was causally important. A study in uninfected rats showed that administration of testosterone indeed decreased the arginine vasopressin promotor methylation and increased the expression of this neurotransmitter (75). Moreover, castration before infection, leading to extremely low testosterone levels, prevented increased attraction to cat urine in infected male rats, while it did not influence the aversion of uninfected rats (39). As discussed above, testosterone levels of infected mice are decreased by *T. gondii* (50), but the fatal attraction phenomenon is nevertheless present in mice (64,65,69), indicating that there is either a testosterone-independent mechanism that operates in mice, or testosterone may not be the direct cause of this behavioral modification in general. Moreover, the effect of the infection on female testosterone levels was inconsistent (42,76), but the decreased aversion to cat odor was reported in both sexes (59,66). In female rats the aversion was dependent on the state of the estrus cycle, and infected females had increased progesterone levels (68); hence it is possible that progesterone may play a role in this manipulation of infected females (67).

Neurological Cysts and Dopamine Hypothesis

Another commonly proposed mechanism for this phenomenon is a preference of the parasite for certain brain regions, leading to local malfunctioning of the brain. This idea arose after two studies in rodents suggested an increased likelihood of *T. gondii* infecting the amygdala (59) and other medial brain structures (77), although both studies also reported tissue cysts in other brain regions and a later study demonstrated large interindividual differences in cyst location and widespread distribution of cysts throughout the brain without selective tropism to any particular functional system (78). Moreover, decreased aversion to cat odor was also observed in infected mice without tissue cysts (69), suggesting no causal relation between the selective tropism of cysts and this behavioral manipulation.

After the discovery that *T. gondii* neurological cysts could enhance the local dopamine concentration (79), it was hypothesized that this causes the decreased aversion to cat odor, since dopamine is involved in motivation (80). In accordance with this view, the genome of *T. gondii* contains two enzymes, AAH1 and AAH2, very similar to tyrosine hydroxylase, which converts L-tyrosine to L-dopa in the rate-limiting step of dopamine synthesis (81). This hypothesis avoids the selective tropism of cysts as a requirement, since neurons outside the endogenous dopamine system will probably not contain dopamine receptors or complementary enzymes for dopamine production (19). However, the expression levels of AAH1 and AAH2 in tachyzoites and bradyzoites are negligible (82). Moreover, *in vitro* and *in vivo* dopamine levels did not differ between uninfected mice and mice infected with either wild-type, AAH2 knock-out or tachyzoites in which AAH2 was upregulated (82).

Neuroinflammation Hypothesis

Recently, it was hypothesized that neuroinflammation could be responsible for this manipulation. This idea arose after several studies described that *T. gondii* infection increased the expression of immune-related genes, including several chemokines and cytokines, and activated microglia and astrocytes in the brain of hosts (64,83). However, infected mice with a transient inflammatory response also exhibited a decreased aversion to cat urine, even when the levels of brain leukocytes were normal again, indicating that continuing brain inflammation was not required for this behavioral change (69).

Additional Hypotheses

In addition to these three main hypotheses, several other hypotheses were proposed, most of them based on the modulation of neurotransmitters (72). However, there are many inconsistencies in the evidence for these hypotheses and none of the supporting studies looked specifically at the fatal attraction phenomenon. Therefore, I will not discuss them here.

In general, the support for each hypothesis seems to be mixed, somewhat contradictory and different across species; hence the exact underlying mechanism of the fatal attraction phenomenon is currently still unclear.

Adaptive Potential

Although there is no direct evidence that infected rodents are more frequently predated upon by felids, the observed decreased aversion to cat odor provides strong indirect evidence of increased rates of trophic transmission into the definitive feline host. Furthermore, mathematical models describing similar trophic transmission of the tapeworm *Echinococcus multilocularis* from its rodent intermediate host to its canid definitive host show that even a slight increase in the susceptibility to predation of intermediate hosts is sufficient for a significant increase in the frequency of the parasite in its definitive hosts (84). Enhanced transmission of *T. gondii* to definitive hosts is very likely beneficial for this parasite. It enables sexual reproduction, which is known to substantially contribute to the genetic diversity of species with a combination of sexual and asexual reproductive strategies, even when the population frequency of sexual reproduction is low (85-87). Sexual reproduction may increase the rate of adaptive evolution to the locally dominant host species, since only strains that have proven themselves to be successful and well-adapted to the intermediate host during the asexual phase, may take part in this sexual phase in which beneficial alleles are exchanged among strains (88). The existence of within-host between-strain competition in *T. gondii* is supported by the observation that multiple strains exist within each infected individual host (89). Furthermore, the generation of oocysts during sexual reproduction enables infection of herbivores, which cannot be infected by tissue cysts via meat consumption (21). Due to their resilience and enormous quantity, these sexually-derived oocysts provide a huge source of infection in the complex life cycle of *T. gondii* (21).

On the other hand, increased trophic transmission of *T. gondii* can interfere with sexual and vertical transmission. When an intermediate host is eaten by a felid, it cannot transmit the parasite anymore to sexual partners or offspring. Mathematical models demonstrate that the degree to which this manipulation is adaptive depends on the relative frequency of vertical and sexual transmission, the virulence in the intermediate host, and other epidemiological dynamics (90). However, especially in virulent strains and epidemic situations, the manipulation is very likely beneficial. Moreover, this study only considered the short-term advantages of the trophic transmission to felids (transmission to new hosts), but not the long-term genetic advantages (increased diversity and more rapid adaptive evolution) of sexual reproduction. Since this can provide substantial benefits for the parasite, as described above, the adaptiveness of the behavioral manipulation may be higher than suggested in this mathematical study.

Decreased aversion of humans to cat odors is not, in the present day, beneficial for *T. gondii*, since the rate at which humans are predated upon by felids is very low. Although evidence for the fatal attraction phenomenon in humans is weak, there are two schools of thought about why this parasite could influence such human behavior. The first states that these behavioral changes in human beings are considered a parasitic constraint, since they are residuals of traits evolved in proper intermediate hosts and humans simply share neurobiological mechanisms affecting the response to feline odor cues with them (91,92). The underlying idea is that *T. gondii* can probably not recognize hosts predated by felids and restrict the infection and behavioral changes to them, leading to accidental infection and manipulation of for example humans. When an oocyte is passively consumed, there is for example no means of the parasite targeting a specific taxonomic group during this point in its life cycle. However, there is growing evidence for an alternative hypothesis that these behavioral changes evolved in early humans that still suffered high levels of feline predation. Since other African primates are significantly predated upon by large felids (93), early humans and their hominid ancestors were probably also under significant feline predation and were thus appropriate intermediate hosts (94). Moreover, the fatal attraction phenomenon was confirmed in our closest living relative, the chimpanzee (95). Infected chimpanzees exhibited a decreased aversion to the urine of leopards, their only natural predator, compared to uninfected animals. Infected chimpanzees did not show this towards the urine of tigers and lions, felids that they do not naturally encounter, indicating that this response evolved exclusively towards coexisting feline predators. In addition, a study in rats showed that *Toxoplasma*-infected animals had a significant preference for the urine of wild cats (cheetahs and pumas) compared to the urine of domestic cats, while uninfected rats had an equal aversion to both (66). The infected rats also moved more slowly in the wild cat zone compared to the domestic cat zone, which would probably increase their predation risk by wild cats under natural conditions even more. Interestingly, oocyst shedding may continue intermittently in wild cats (96 cited by 97), while domesticated cats only shed oocysts for 20 days after acquiring the infection (21). These results suggest that wild cats may be superior definitive hosts and/or have had a stronger or longer coevolution with *T. gondii* compared to domestic cats, and that human beings may have been a relevant prey animal of such species (66).

Effects on the Secondary Sex Ratio

Toxoplasma infection is also thought to influence the secondary sex ratio (SSR, expressed here as the chance of giving birth to a son, normally 0.5). A study among women in Prague revealed that the SSR was strongly influenced by the infection, with the effect depending on the amount of anti-*Toxoplasma* antibodies (98). The SSR was significantly male-biased (0.72) for women with the highest indirect immunofluorescence test (IIFT) titres of over 128, but significantly female-biased (0.40) for women with the lowest levels of these antibodies (IIFT titre 16). Presumably, women with high titres had much more recent infections than women with low titres (99), since anti-*Toxoplasma* antibody levels decrease over time (100). Similar results were obtained in a study of umbilical cord blood samples in Teheran (101), where the odds of having a son was nearly 64% higher for *Toxoplasma*-infected women than for uninfected women and 110% higher for infected women with high antibody titres and presumably more recent infections (optical density over 0.75). This pattern was confirmed by a study in female mice (14). Orally infected mice had a significantly higher SSR than controls when they delivered 86 to 120 days after being infected (for two experiments respectively, 0.66 and 0.55 in infected females compared to 0.32 and 0.46 in controls), but a significantly lower SSR when they delivered 121 to 222 days after being infected (respectively, 0.41 and 0.38 in infected females compared to 0.59 and 0.55 in controls). The magnitude of this manipulation by *T. gondii* in human beings was demonstrated by an ecological regression study across 94 populations worldwide (102). The prevalence of the infection appeared to be the third most important predictor of the SSR. Moreover, the correlation between this prevalence and the SSR was negative, which is in accordance with the previous studies (99). Since in high-prevalence countries women are more likely to get the infection early in life, they likely already have a chronic infection when they give birth (99), which is associated with a low SSR. However, in low-prevalence countries the chances of early infection are much lower and women are more likely to be infected close to conception (99,103), which is associated with a high SSR.

Proposed Mechanisms

Testosterone Hypothesis

The first explanation for the effects of *T. gondii* on the SSR arises from the idea that high levels of paternal and maternal testosterone are associated with a high SSR (104). Increased testosterone levels are found in infected males, as previously described (99). However, in order to explain the observed patterns, this would imply that infected males impregnated more frequently recently infected females (or uninfected females while sexually transmitting *T. gondii*) than females with an old infection, since the SSR was negatively correlated with the age of the maternal infection. This seems unlikely and could not have been the case in the abovementioned study of the SSR in mice. As previously described, the effect of the infection on female testosterone levels was inconsistent, but there were no indications that the age of infection differed between the studies. Moreover, female mice exhibited reduced testosterone levels two months after infection compared to controls (50), which conflicts with the aforementioned study showing that the SSR in mice was male-biased at that time since acquiring the infection.

Immunosuppression Hypothesis

Another proposed mechanism for the overproduction of males by recently infected females is *Toxoplasma*-induced immunosuppression. During pregnancy the sex ratio fluctuates due to male- or female-biased mortality, eventually resulting in a slightly male-biased sex ratio at birth (105). Several studies suggest that a maternal immune response against male-specific H-Y antigens encoded by the

Y-chromosome causes a portion of deaths of male embryos (106-108). Therefore, suppression of the maternal immune system may increase the survival of male embryos, resulting in the observed male-biased SSR. Immunosuppression after *T. gondii* infection was demonstrated by a study in mice (109). During the course of the infection, the infection-induced production of IL-12 declined, while the production of the immunosuppressant cytokine IL-10 increased. Moreover, the production of pro-inflammatory NO in macrophages and IL-2 and IL-4 in spleen cells was decreased in infected compared to uninfected animals.

Trivers-Willard Hypothesis

The decreased SSR in women with old infections could be explained by the Trivers-Willard hypothesis, which states that mothers in good condition tend to produce more sons and mothers in poor condition more daughters (110). It is based on the idea that natural selection will select for a maximized expected reproduction by the offspring and that the variance in fecundity (reproductive skew) is much higher for males than females. Males in good condition have a higher expected reproductive output than their sisters, since they may mate with multiple females. However, males in poor condition have a lower expected reproductive output than their sisters, since unlike females they may be outcompeted and end up with no mates at all. The reproductive output of female offspring, being less skewed, is more dependable than that of males under poor conditions. This hypothesis has been confirmed by several studies in humans and other mammals (111,112). It is suggested that long-term negative effects of *T. gondii* on the host's health lead to a female-biased SSR in women with old infections (14). The underlying mechanism is probably the blood glucose level of the mothers, which positively correlates with their condition, since an excess of glucose supports the development of male blastocysts and inhibits the development of female blastocysts during early cell division (112). This is consistent with a retrospective study of pregnant women in Prague demonstrating a positive correlation between their level of anti-*Toxoplasma* IgG antibodies and their blood glucose 120 minutes after a glucose drink (113). Since the amount of these antibodies decreases when the infection is older (100), this suggests that the blood glucose of these women declined over time since first infection, perhaps reflecting the health effects of infection, leading to a female-biased SSR according to the Trivers-Willard hypothesis.

Adaptive Potential

Generating a male-biased SSR in recently infected mice could be an adaptive mechanism of *T. gondii* to enhance its transmission. Since in most rodent species, males exhibit more exploration (114), have larger home ranges (115), show more aggressive behavior (116) and therefore carry out more geographic dispersal (117) than females, production of more male offspring may facilitate long-range transmission of the parasite (98). An increased SSR may also enhance trophic transmission, since male rodents show less fear and avoidance of predators than females (118) and are more frequently predated upon (119).

It has been argued that females should nevertheless be more valuable for *T. gondii* transmission than males, since they can transmit the infection vertically (99). However, the time period in which vertical transmission can take place is, as described above, almost exclusively in recent infections. The male-biased SSR when the parasite is vertically transmissible can be adaptive, since congenital infection of females prevents them from vertically transmitting *T. gondii* themselves due to the age of their infection (14). Therefore, congenitally infected males and females would be equally valuable for *T. gondii* concerning vertical transmission (since they are both not capable of that), but males could be more valuable for its long-range and trophic transmission.

Discussion

I have described three types of potentially adaptive behavior modification that take place in intermediate hosts during *T. gondii* infection: increased male attractiveness, the fatal attraction phenomenon and adjustment of the secondary sex ratio. Evidence for these effects is drawn from studies of rats, mice and humans, and the effects are not always consistent across these species. The parasite has a single definitive host, but it infects a very wide range of intermediate hosts, since, unlike many parasites, it is probably unable to restrict infection to appropriate intermediate hosts at least during the oocyte stage of its life cycle. The hosts probably differ in the mechanisms they use to evaluate the attractiveness of mates, assess odor cues, and possibly in the way they regulate the secondary sex ratio. Therefore, the behavioral consequences of the manipulation might not be the same in different host species. Exposure of the parasite to many diverse intermediate host species may prevent the evolution of specialized patterns of behavioral modification that are consistent across all host species. In particular, there may be disruptive or fluctuating selection depending on the presence or absence of potential intermediate host species, in which the behavioral modifications are only effective in the most common hosts, in which they are adaptive in some hosts but maladaptive in others, or in which they are suboptimal in all hosts.

Since many humans are infected by *T. gondii*, the effects of the infection on human behavior are of popular interest. Although all three behavioral manipulations are supported by much evidence in rodents, only the manipulation of the SSR by *T. gondii* is well-studied and confirmed in humans. The other two manipulations are only supported by one study providing indirect evidence for the increased attractiveness of men and one study providing conflicting evidence for presence of the fatal attraction phenomenon. Therefore, the presence of these behavioral manipulations in humans is not settled and should be confirmed by more research, before one can determine whether the underlying mechanisms that operate in rodents are also active in humans.

Concerning those mechanisms, it stands out that increased testosterone levels are proposed as mechanism for all three phenomena. This idea is very appealing according to Occam's razor (121). However, while the fatal attraction phenomenon and the temporally opposite effects on the SSR are also present in mice, increased testosterone levels are not, although this is only supported by one small study. The effect of the infection on female testosterone levels is also understudied and inconsistent. Therefore, I want to stress the need for more research on testosterone levels of infected mice and females in order to exclude or confirm testosterone as mechanism for these phenomena. Furthermore, it is essential to study the effect of infection on testosterone in a broader range of potential rodent hosts such as voles and squirrels so that it can be determined whether it is the rat, or the mouse, that is unusual.

Although none of the proposed mechanisms for the fatal attraction phenomenon is supported unequivocally by empirical evidence, the testosterone hypothesis would be the most likely mechanism due to the impressive support for it in male rats and the possible extension of this mechanism to females through a potential effect of progesterone. However, the main challenge of addressing the proximate causes of the fatal attraction phenomenon will, in my opinion, be explaining its specificity. How does *T. gondii* only affect the aversion of its intermediate host to the odor of felids? This specificity seems to imply distinct neural pathways or substrates for processing feline odors that must be present across phylogenetically diverse taxa including both rats, mice and human beings (59). More research on feline odor processing in the intermediate hosts of *T. gondii* could help determine how this parasite specifically affects this pathway.

Furthermore, the three proposed mechanisms for manipulation of the SSR are not mutually exclusive and may operate together. Since testosterone is known to induce immunosuppression (51), these two mechanisms may be synergistic and underly together the increased SSR in recently infected females. As the condition of the host decreases over the course of the infection, the opposite effect of the Trivers-Willard hypothesis on the SSR may eventually outweigh this, leading to a decreased SSR (14). However, the evidence that these mechanisms underly this modification is still only indirect.

The temporal resolution of these phenomena is also understudied. This is especially important since the presence of different cellular stages of *T. gondii* is restricted to certain phases of the infection. Tachyzoites, responsible for sexual and vertical transmission, are for example only present during the acute phase (23,24). Since the adaptive potential of the male attractiveness and SSR manipulation depend on these forms of transmission as described above, determining the temporal resolution of (the mechanisms underlying) these manipulations would be valuable. However, since tissue cysts remain present in the long term even when antibodies are no longer detectable and human studies depend on antibodies as indication for the presence and age of the infection (91,122), this resolution can only be properly studied in non-human animals.

As described previously, host-parasite interactions are often considered an evolutionary arms race, which can be described using the metaphor of the Red Queen who must continually keep running in order to stay in the same place (1,2). Considering the substantial behavioral changes in the intermediate host, at least in rats, it is tempting propose that *T. gondii* has actually won the evolutionary arms race. However, I want to argue to the contrary that the Red Queen hypothesis can still be applied to this particular host-parasite interaction. Since there is likely a strong selective pressure on the intermediate host to avoid the fatal attraction phenomenon in particular, *T. gondii* still has to evolve as fast as possible to keep its manipulative ability and stay constantly one step ahead (1).

The adaptiveness of host behavioral manipulations by parasites was critically reviewed by Poulin in 1995 (3). It was argued that manipulations should only be regarded as adaptive for the parasite if they meet the following criteria: complexity, indications of purposive design, convergence in evolution and causally increased fitness of the parasite. Therefore, I will discuss whether the adaptive potential of the three manipulations described in this thesis satisfies these criteria and which knowledge gaps need to be filled in order to assess them. Since the described adaptive potential in humans is smaller than in rodents, I will discuss this for rodents and subsequently argue to what extent this is applicable to humans. However, it should be mentioned that these criteria are not strict, since manipulations that appear simple can nevertheless have complex underlying mechanisms and tiny fitness advantages can be selected over a long period of time or in very large populations (3). The criterium of purposive design also needs some explanation, since evolution, of course, does not have a purpose. It just states that a trait too well fitted to its function to have arisen by chance reflects the shaping force of selection (3).

The adaptive potential of the increased attractiveness of infected males seems to meet all four criteria. Complexity of the manipulation is implied by its influence on the behavior of uninfected animals, the females (35). Purposiveness of design is suggested by the sexual transmission of *T. gondii* and the female preference for infected males, which conflicts with their natural avoidance of parasitized males (52-54). Analogous increased host attractiveness to enhance sexual transmission is observed in corn earworm moths after infection with the insect virus *Hz-2v* (123). Moreover, the increased mating success of *Toxoplasma*-infected males and demonstrated sexual transmission of this parasite, provide strong evidence for increased transmission of this parasite as a result of the increased attractiveness. Since increased sexual transmission does not interfere with vertical or trophic transmission, it will very likely increase the fitness of *T. gondii*.

When applied to the fatal attraction phenomenon, it can be argued that at least the first three criteria are met. The manipulation seems complex since it appears to be specific for feline odors by an unknown but highly targeted mechanism, which in combination with the restriction of sexual reproduction to felids also suggests purposiveness of design. Moreover, analogous manipulations are observed in several other host-parasite interactions (7-9). But, despite the strong indirect evidence for increased fitness of *T. gondii* by this manipulation, the direct evidence is still missing. Although a traditional predation experiment would be difficult due to ethical issues (124), monitoring the survival of infected and uninfected rodents in their natural environment in a capture/recapture experiment to determine whether infected animals are more frequently predated by felids should be possible. Moreover, extending existing mathematical models (with for example long-term benefits of the parasite's sexual reproduction) and integrating them with data of such a survival monitoring experiment, would help determine the conditions under which the fitness of *T. gondii* increases by enhanced feline predation on intermediate hosts (71). This could generate testable hypotheses about the likelihood of this phenomenon in *T. gondii* strains with for example different amounts of vertical and sexual transmission or virulence (71). Experimental confirmation of such predictions would establish the adaptiveness of the fatal attraction phenomenon even more (71).

Support for the adaptiveness of the temporally opposite effects of *T. gondii* on the SSR is mixed. Although complexity of the manipulation could be argued by the effects being temporally opposite, the underlying mechanisms seem to be simple side-effects of infection (Trivers-Willard hypothesis, 14) or selected for due to other benefits for the parasite (enhanced testosterone and immunosuppression, 99). Purposiveness of design could be suggested by the male-biased SSR when the infection is vertically transmissible in combination with the more migratory character and higher predation rate of males. Moreover, adaptive manipulation of the host's SSR is reported in the parasite *Wolbachia*, confirming convergent evolution (125). However, evidence for increased fitness of *T. gondii* by SSR manipulation is weak, since it is indirect and mainly based on speculations (14,98).

Although there is growing evidence that human ancestors may have been appropriate intermediate hosts for *T. gondii*, modern humans seem to break its life cycle. The parasite becomes stuck in this host, due to inhibited trophic transmission by the lack of predation on modern humans. Whether or not the fatal attraction phenomenon is present in modern humans, they have won the evolutionary arms race with *T. gondii* concerning this manipulation by using the ultimate weapon: excluding feline predation. Moreover, the lack of trophic transmission may lead to the evolution of another kind of parasite in modern humans, since selective pressures on *T. gondii* related to predation and transmission between intermediate and definitive hosts are removed (126). Once *T. gondii* has entered a human being, only horizontal (sexual) and vertical (pregnancy-related) transmission are left. This implies that the strains of *T. gondii* present in human populations only ever reproduce asexually and never generate oocysts, a very different scenario to the one that has held during most of its evolutionary history. One would expect the evolution of reduced virulence over time due to the requirement of a healthy living host for these forms of transmission (126,127). Although increased attractiveness of infected males is understudied in humans, this manipulation would probably be adaptive for *T. gondii* in modern human populations based on similar reasoning as in rodents and the increased dependency on sexual transmission in human hosts. The adjustment of the SSR by the infection is confirmed in humans. However, genetic analysis reveals that the human migration rate is higher for females, probably due to the spread of agriculture leading to patrilocality (128). In combination with the relative unimportance of predation to human mortality, the indirect evidence from rodents for the adaptiveness of this manipulation will not hold for modern humans.

Conclusion

In conclusion, there is much evidence for the increased attractiveness of males, the decreased aversion to cat odor and the adjustment of the SSR after *T. gondii* infection in rodents. However, only the last phenomenon is well-studied and confirmed in humans. The underlying mechanisms of these three manipulations are not completely clear. While the adaptiveness of especially the first two behavioral manipulations is supported by strong indirect evidence in rodents, only the second manipulation has the potential to be adaptive in modern humans.

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