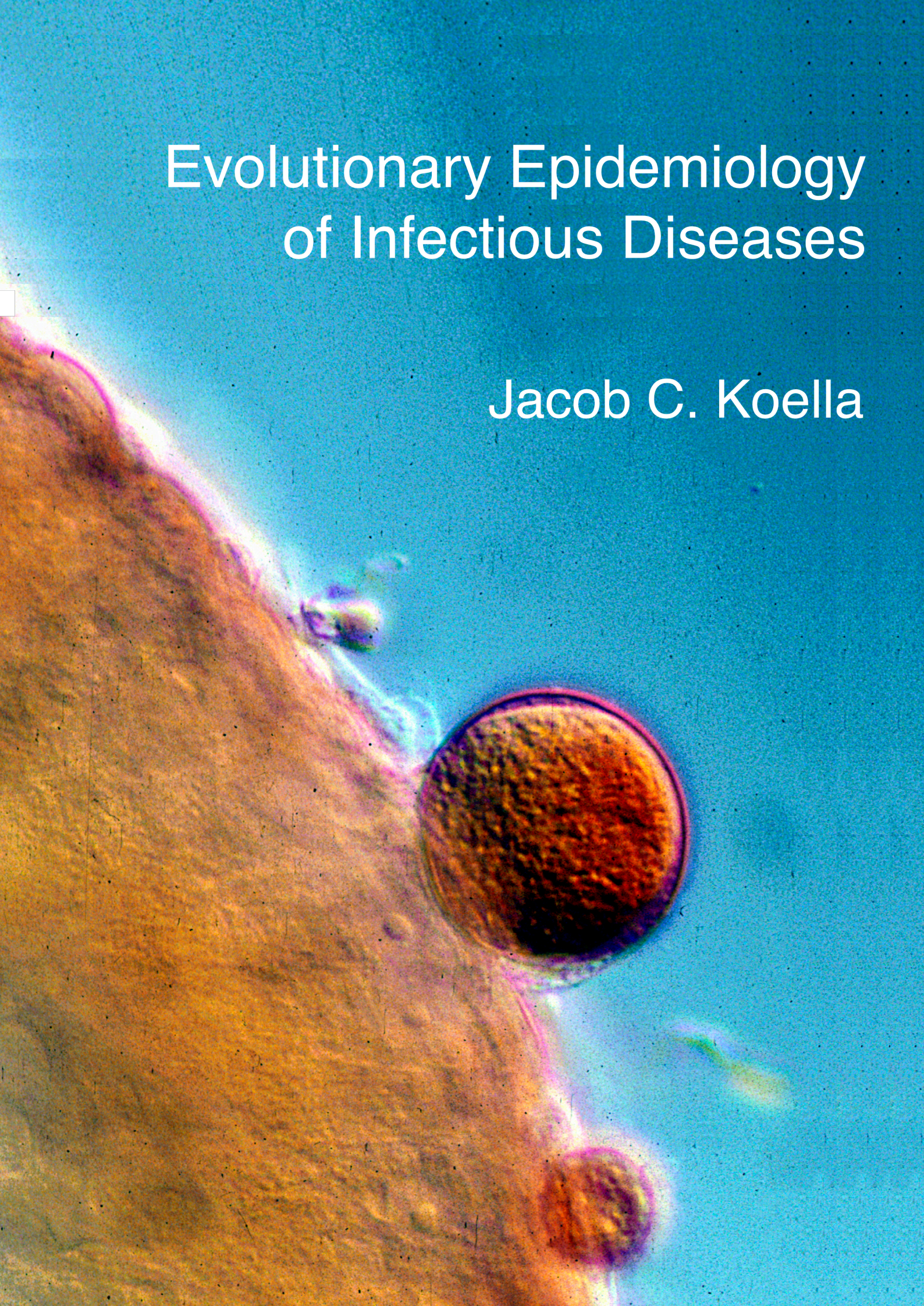
Evolutionary Epidemiology of Infectious Diseases

Jacob C. Koella



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Manipulation

We all want to believe in free will, that we can choose our own behaviour. Scientists trying to understand an individual's behaviour, let's say with whom a female mates, will usually assume that their study organisms can control their behaviour, that the female chooses her mate according to her preferences, that she chooses a mate to have more, and more successful offspring. But this assumption may often be wrong. It turns out that the behaviour of many organisms is controlled by their parasites, to the advantage of the parasite rather than the host.

Cordyceps, mentioned in the introduction, is one example of such manipulation that enhances the parasite's transmission. The fungus takes over the ant's behaviour, making it crawl up a plant and attaching itself to it. From high above the ground the parasite's spores can transmit to a larger area, making it more likely that they can be encountered by other hosts. The bacterium that causes the plague (also mentioned in the introduction) and the protozoan that causes sleeping sickness are two others. Both make their insect vectors – fleas for the plague, tsetse flies for sleeping sickness – bite more frequently by making it more difficult for them to obtain their blood meal. Let's look at a few examples in more detail.

The jewel wasp (*Ampulex compressa*) uses cockroaches as brooding bodies for her offspring: a female lays an egg into the cockroach, the larva develops within it and eventually kills it, and the wasp emerges from the cockroach as a fully developed adult. This is all normal parasitoid behaviour. What makes this system special is that the jewel wasp buries the cockroach alive and that the cockroach remains in its future tomb alive and capable of moving, but without trying to escape. It is of course safe to assume that it is not the cockroach that is in control of this behaviour. The mechanism by which the wasp takes control is a sophisticated feat of neurosurgery. When a wasp encounters the cockroach, she stabs it with her stinger, targeting the part of the nervous system that controls the cockroach's walking rhythm. This first sting temporarily paralyses the cockroach, giving the wasp the time to sting its victim a second time; this time she injects a venom into a specific cluster of nerve cells near the brain. The venom doesn't kill, paralyse or even sedate the cockroach; all it does is remove its motivation to walk. It therefore does not resist when the wasp leads it into a burrow by pulling on an antenna, where she lays an egg into the body and then covers the burrow. The cockroach remains patiently inside the burrow even while it is being devoured alive by the wasp's descendant. (Several websites show movies of this behaviour; have a look at this one[1] or this one[2].)

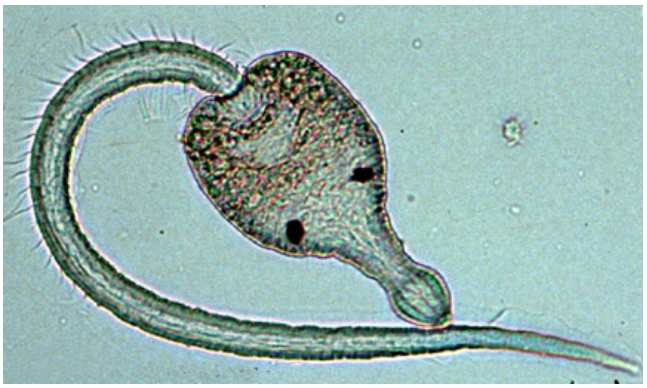
[1] https://www.youtube.com/watch?v=-ySwuQhruBo
[2] https://www.youtube.com/watch?v=vl_9kghmChw

Left side: A jewel wasp emerging from the carcass of a cockroach, within which the wasp has completed its larval development. Right side: A caterpillar defending the pupae of the wasp *Glyptapanteles*.

Another wasp, called *Glyptapanteles*, lays many eggs into a single host, a caterpillar. Again, the developing larvae eat the caterpillar alive until they finally emerge to form cocoons, the pupal stage of the wasp. But in this case, the host doesn't die. Rather, a few of the wasps stay behind, inside of the caterpillar, to manipulate the its behaviour. It now stands guard over the siblings of the manipulators - the cocoons within which the wasps are metamorphosing into adults - and attacks any other insect approaching. The wasp has turned the caterpillar into a bodyguard defending the parasites that have robbed it of its future. Have a look at a movie of the behaviour here[3].

You may have seen a cricket jumping into a pool of water in an apparent attempt to kill itself. Silly behaviour? Well, not for the nematomorph, *Spinochordodes nematoporhp*, parasitising it. Like all parasites, nematomorphs must solve the problem of getting to a new host, but for nematomorphs this is more difficult than for most, for it cycles between infecting a terrestrial and infecting an aquatic animal. The female lays her eggs in water, where her offspring infect an aquatic insect, often the larva of an individual that will move to land as an adult. If the parasite is lucky, its host will therefore emerge and then die on land, and the parasite may then be ingested by a cricket, its second host. The worm will grow until it completely fills the cricket's body cavity. But, how can it now get back into the water to complete its development and lay eggs? Since crickets avoid water, this might appear unlikely, but the parasite changes several proteins in the cricket's brain to make it seek out and jump into water (where it will usually drown). The cricket thus sacrifices itself to help its parasite to complete its life-cycle. Here is a movie of the behaviour[4].

[3] https://www.youtube.com/watch?v=6lk_m1AF4Xk
[4] https://www.youtube.com/watch?v=D7r1S6-op8E



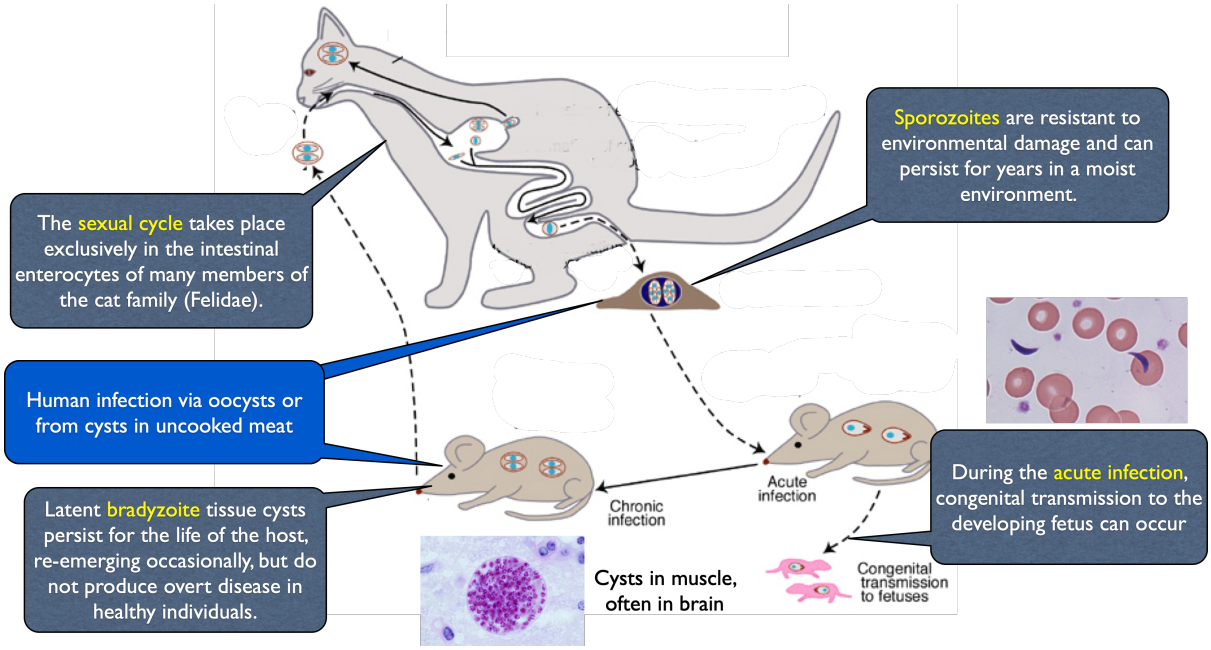
A cercaria of the trematode *Euhaplorchis californiensis*.

Don't think that only insects are manipulated. The creature above is one of the developmental stages (called cercariae) of the trematode *Euhaplorchis californiensis*. This parasite has a complex life cycle that involves three hosts. It begins its development as a miracidium, which infects (as do all trematodes) a snail. Once inside the snail, the parasite replicates extensively and eventually produces thousands of cercariae. When these are shed from the snail, they go on to infect a killifish (*Fundulus parvipinnis*), in which the parasites develop into metacercariae that encyst in the brain of the fish. If the fish is eaten by a bird, the parasites continue their development into adults and lay eggs, which are passed with the bird's faeces into the water to hatch into miracidiae that start a new cycle. You can imagine that the transmission from fish to bird is problematic, as killifish generally try to avoid being eaten. Once again, the parasite increases its chances by taking over the control of its host's behaviour. The metacercariae change the concentrations of dopamine and serotonin in the brain to reduce the stress response of the fish and to make them swim conspicuously, thrashing in jerky movements at the surface of the water. This makes them an obvious and easy target for birds; it lets birds catch and consume infected fish 30 times more frequently than uninfected ones [5].

Our next parasite is a protozoan, the apicomplexan *Toxoplasma gondii*. You may have heard of this parasite; you may indeed be carrying it in your body, for it infects about 30% of the world's population (though prevalence varies widely among countries from a few percent to about 70%). It doesn't cause many acute symptoms, but if a woman is newly infected during her pregnancy, it can cause still-births or severe neurological problems and blindness of her baby. Luckily it is fairly easy to avoid being infected with simple hygiene measures: washing hands, washing vegetables, cooking meat, or avoiding contact with cats. (You will understand how this helps in a minute.) We are considered accidental hosts of *Toxoplasma*, and indeed when we are infected, the parasite will have a hard time completing its life cycle. To do so, the parasite would have to get into its final host, a cat. This is the only host that lets the parasite reproduce sexually. Eventually parasites are passed out with the cat's faeces, and wait to be eaten by a potential intermediate host, which can be any one of a number of mammals, a mouse or a rat, for example, but also sheep and cattle. After some initial replication the parasite encysts in various tissues, including the brain. Humans become infected by eating vegetables contaminated with the parasite, eating the uncooked meat of an infected intermediate host, or by picking up the parasite by contact with a cat. Once inside the mouse, the parasite can be transmitted in three ways. If the parasite is in a female mouse (or other intermediate host), it can be transmitted vertically to the developing

[5] Lafferty , K.D. & A.K. Morris. Altered behaviour of parasitized killifish increases susceptibility to predation by bird final hosts. Ecology 77:1390-1397.

foetus. It is this vertical transmission that causes the severe health problems in humans, for the foetus has effective immune response against the parasite. Transmission also occurs if the mouse is eaten by a cat or, if an infected male mouse transmits it sexually to its partner. *Toxoplasma* manipulates both latter routes of transmission.

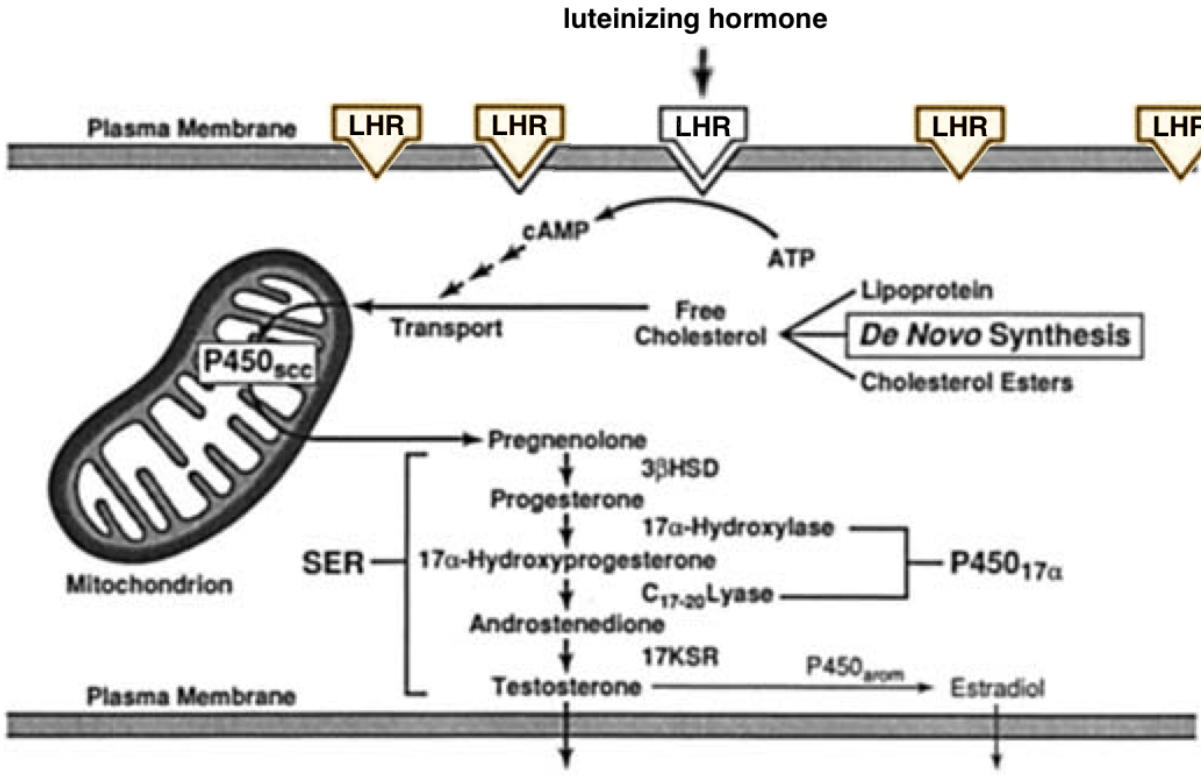


Life cycle of *Toxoplasma gondii*.

One would predict that a female mouse would avoid mating with an infected male. If she is infected, the consequences are as severe as for us: the parasite will infect and likely kill her offspring. Nevertheless, if given a choice a female mouse *prefers* infected over uninfected males as mates; she seeks out the partner that will kill her offspring. She does this because she is being (indirectly) manipulated by the parasite, which stimulates the production of testosterone in the male, turning it into a sexually attractive supermouse [6]. Now, the synthesis of a testosterone is a complex biochemical process, starting with the recognition of luteinizing hormone by luteinizing-hormone-receptors (LHR) and ending with the reduction of androstenedione to produce testosterone. *Toxoplasma* enhances this synthesis simply by masing males produce more of the receptor to enhance the rate of the whole process [7].

[6] Dass S.A.H. et al. 2011. Protozoan parasite *Toxoplasma gondii* manipulates mate choice in rats by enhancing attractiveness of males. PLoS ONE 6:e27229.

[7] Lim, A. et al. 2013. *Toxoplasma gondii* infection enhances testicular steroidogenesis in rats. Molecular Ecology 22:102-11.



Schema of production of testosterone.

Whether male or female, the sensible thing for a mouse to do is avoid being eaten by a cat. However, infected rats not only lose their natural fear of cats, they are actually attracted to the scent of cat pee. Indeed, the parasite tricks its host into being sexually aroused by the scent. A true fatal attraction….[8]

Now, mice are mammals and the intermediate host of this parasite; we are also mammals and the equivalent of the intermediate host (even if we are not often eaten by cats). So, could the parasite also manipulate our behaviour? You may not want to believe it, but there is some evidence that it indeed does. Infected people are more than twice as likely to be involved in car accidents as uninfected ones, perhaps because the enhanced synthesis of testosterone makes them loose some of their sense of fear [9]. (I'd like to check the rate of infection among bungee-jumpers and base-jumpers.) They also score differently on personality questionnaires. Furthermore, *Toxoplasma* may underly some cultural differences among countries: those countries with a higher prevalence of toxoplasmosis score higher on NEO-PI-R (the standard for personality assessment) for neuroticism, which combines, among others, anxiety, hostility, depression and impulsiveness, and they have higher rates of suicide [10].

[8]Berdoy, M., J.P. Webster & D.W. Macdonald. 2000. Fatal attraction in rats infected with *Toxoplasma gondii*. Proceedings of the Royal Society B: Biological Sciences 267:1591–1594.

[9]van Honk J. 2005. Testosterone reduces unconscious fear but not consciously experienced anxiety: implications for the disorders of fear and anxiety. Biological Psychiatry 58:218-25.

[10]Lafferty, K.D. 2006. Can the common brain parasite, *Toxoplasma gondii*, influence human culture? Proceedings of the Royal Society B 273:2749-2755.

Is manipulation good for the parasite?

While in these examples it seems clear that parasite increases its transmission by manipulating its host's behaviour, the situation is not always so obvious. Indeed, you may even ask why *Glyptapanteles* manipulates caterpillars to guard the offspring. Of course, from the mother's point of view, manipulation is good: most of its offspring are guarded and are therefore more likely to survive. But from the offsprings' point of view, the situation is not so clear. Why do some individuals stay behind to control the caterpillar's behaviour, and thereby sacrifice themselves for the good of those that are guarded? In this particular case, the answer is simple. Since the wasps that are left behind are siblings of those that escape and are guarded, this is a case of kin selection. Siblings share 75% their genes, so slightly increasing the survival of many siblings may let a manipulator pass more of its genes to future generations than ensuring its own survival. (Remember that wasps are haplodiploid; in a diploid species sisters only share 50% of their genes.)

Kin selection

A similar argument may hold for a classical case of manipulation: the trematode *Dicrocoelium dendriticum* (also called the liver fluke). The adult parasite is found in the liver and bile duct of grazing mammals such as sheep and cows. It lays eggs that are shed with the faeces and turn into miracidia. If a snail eats the faeces, it becomes infected. The miracidia hatch, drill through the wall of the gut and settle in the digestive tract to replicate and eventually produce cercariae. The snail, trying to protect itself, encysts and excretes the cercariae. Ants are attracted to the trail of snail slime, and swallow cysts that are loaded with hundreds of cercariae. The parasites then become active migrate through the ant's body, and encyst in a part of the ant's brain that is next to the mandibular nerves. These cysts start manipulating the ant's behaviour[11]. Every evening (when grazers are most active), infected ants climb up a blade of grass, and clamp their mandibles onto the top of the blade because of temporary tetanus caused by the infection. At dawn, they go back to their normal activity in the ant colony. This is repeated until a grazing animal eats the blade, thereby ingesting the ants and the parasites together with the plant.

Left side: Adult liver fluke. Right side: Ant with tetanus clamping down on a blade of grass.

[11] https://www.youtube.com/watch?v=lGSUU3E9ZoM

So far, this has been the classical story. But only one parasite migrates to the brain, the rest stay in the hemocoel. The parasite in the brain is the only one responsible for the manipulation. It will not complete its development, it will not infect the final host, and it dies when the ant is eaten. Thus, similar to the case of *Glyptapanteles*, the parasites that do not manipulate profit from the one manipulator and are transmitted, while the manipulator sacrifices itself for the others. And like in the case of *Glyptapanteles*, this appears to be a case of kin selection. The cercariae that are eaten by an ant most likely come from a single infection in the snail. The cercariae in an ant are therefore likely to be genetically identical, which makes it evolutionarily worthwhile that a parasite sacrifices itself for the good of many members of the same clone.

What if there is no kin selection?

The situation is not always so easy to explain. Let's look at yet another trematode, *Microphallus papillorobustus*, which has a snail (of course, as this is a trematode), a shrimp (*Gammarus insensibilis*) and birds in its life-cycle. The cercariae released by the snail infect the shrimp, encyst as metacercariae in its brain, and then wait until the shrimp is eaten by a bird, where the parasite develops into an adult. The usual story is similar to that of *Euhaplorchis* infecting killifish: the metacercariae, which have encysted in the brain, change the behaviour of their host and make it swim upwards, making it more vulnerable to predation and increasing their transmission[12].

But only some parasites encyst in the brain, and these are responsible for the manipulation; the others encyst in the abdomen and are freeloaders of the manipulators. Making things worse for the manipulators, the parasites in the brain are more likely to die than the freeloaders. Shrimps have effective immune responses, and these target, in particular, the metacercariae in the brain and kill about 17% of them; of the cysts in the abdomen, the snail kills only 1% [13]. Thus, the parasites in the abdomen profit from the manipulators without suffering much of the burden of the immune response. Now, since the cercariae of this parasite are free-swimming and therefore infect shrimp independently of each other, there is no reason to assume that the metacercariae in a shrimp are related; this is no simple case of kin selection. So, what is going on? Why do some parasites seemingly sacrifice themselves for the good of the others?

To understand this system, let's estimate with a crude model the evolutionary success of a manipulator and that of a freeloader. We assume that there is a frequency p of manipulators in the population, so a frequency (1-p) of freeloaders. Therefore, if the cercariae infect shrimps randomly and there are N metacercariae in a shrimp, the probability that at least one of them is a manipulator is $f = 1 - (1 - p)^N$. We further assume that one manipulator is enough to control the behaviour of the shrimp, and that manipulation increases the probability that a parasite is transmitted from a level T to a level $T + \epsilon(1 - T)$. (I calculate this expression by assuming that manipulation decreases the risk of *not* transmitting to $(1-T)(1-\epsilon)$, letting the value of ϵ be bound by 0 and 1.) Thus, if ϵ is 0, there is no manipulation; if it is 1, the the shrimp is certainly eaten and the parasite is certainly transmitted. We assume that the mortality due to the immune response is μ in the brain and, to simplify, 0 in the abdomen. Finally, we assume that one manipulator is enough to manipulate the host's behaviour, and that the strategy – to manipulate or not – is fixed in a parasite.

[12]Ponton, F. et al. 2005. Ecology of populations parasitically modified: a case study from a gammarid (*Gammarus insensibilis*) - trematode (*Microphallus papillorobustus*) system. Marine Ecology Progress Series 299:205–215

[13]Thomas, F. E. Guidner & F. Renaud. 2000. Differential parapet (Trematoda) encapsulation in *Gammarus aequicauda* (Amphipoda). Journal of Parasitology 86:650-654.

We can now calculate the probability that a given parasite will be transmitted, if it happens to be in a host with another N parasites. If the parasite is a manipulator its success is simply the product of its survival and the enhanced transmission

$$W_M = (1-\mu)(T+\epsilon(1-T))$$

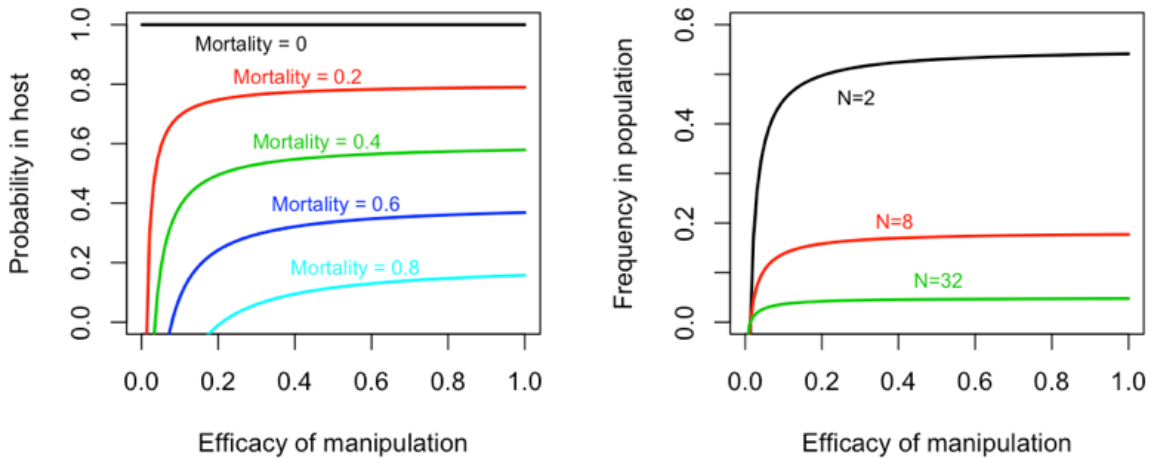
If is is a freeloader, its success depends on whether any of the other parasites are manipulators:

$$W_F = f(T+\epsilon(1-T)) + (1-f)T = T + f\epsilon(1-T)$$

We should expect that the parasite will manipulate its host, if it can expect greater success as a manipulator than as a freeloader, so if $W_M > W_F$, that is if

$$f < 1 - \mu\left(1 + \frac{T}{\epsilon(1-T)}\right)$$

Thus, despite the cost of manipulating (the probability that the cysts in the brain are killed by the immune response), it is a benefit to manipulate the host, if other manipulators are sufficiently rare. If, however, manipulators are frequent, a freeloader can profit from their manipulation without suffering the cost. The figure below shows for different intensities of infection and for different values of cost (μ) and benefit (ϵ) of manipulation the curve where the success of manipulators equals that of the freeloaders. Above the line, freeloading would be better than manipulation; below the line manipulation would be better than freeloading. The line therefore gives the frequency of manipulating parasites we expect in the population.



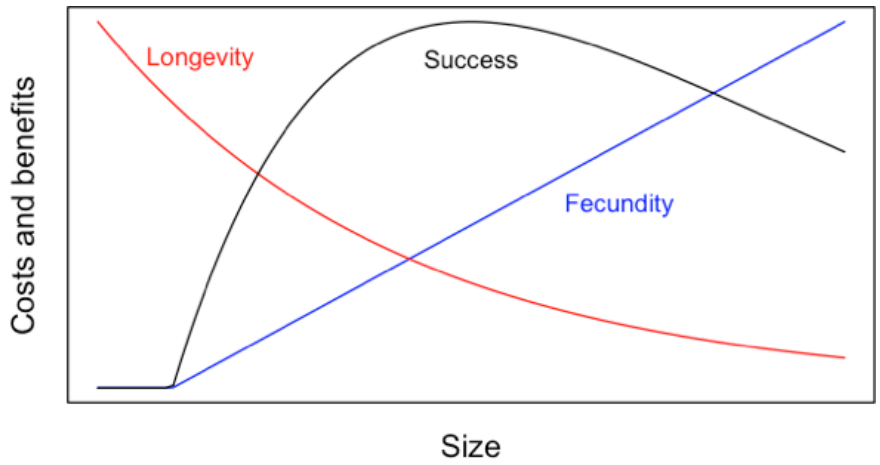
Results of a crude model of the transmission of *Microphallus papillorobustus*, showing the conditions that enable a manipulator to have higher transmission success than a freeloader. The probability, T, of being transmitted from an unmanipulated host is set to 0.05. In both panels the curves show the frequency of a manipulating parasite below which it pays to be a manipulator panel. Left-hand panel: The frequency of manipulators within a host, for different levels of immune-induced mortality (i.e. cost of manipulating). Right-hand panel: The frequency of the manipulator in the population of parasites, for several intensities of infection (that is, numbers of parasites within a single host individual.)

The idea so far is that *evolution balances the costs and benefits of manipulation* (whereby the benefits can be for the manipulator itself or for its genetic relatives). This is the fundamental

idea underlying evolution, so let me repeat it with an example; let's look at (the caricature of) the evolution of body size.

The currency of evolution, in which we express costs and benefits, is the number of descendants (which, for a parasite, is the number of hosts a parasite is transmitted to). As illustrated in the figure below, I assume that being big has benefits: at any given time large individuals can produce more offspring than small ones. But I also assume that being big is costly: large individuals may be preferred by predators, so they live less long than small ones. What matters for evolution is the number of offspring over the life-time of the organism, the *life-time reproductive success*, which in this simple case is the product of fecundity (per time unit) and longevity. Now, a small individual would benefit by being larger, and this benefit would be greater than the cost. A slightly larger individual would have greater life-time reproductive success, so evolution would push body size up. However, for a large individual the cost of being even bigger would outweigh the benefit. Indeed, although it would produce fewer offspring per time if it were smaller, it would suffer less from the cost of being large, so its life-time reproductive success would be greater. Overall, evolution will tend to move traits toward the optimal size, where life-time reproductive success is greatest, where the benefits and costs of being large are balanced.

More quantitatively, let's say that fecundity F as a function of size S is defined by $F(S)$, and longevity L is defined by $L(S)$. Life-time reproductive success is $F(S)L(S)$. It is maximal if $F'L + FL'=0$ (where the prime ' denotes the derivative with respect to size), that is, when the (relative) benefit F'/F is equal to the cost $-L'/L$. (Note that the derivative to longevity is negative.)

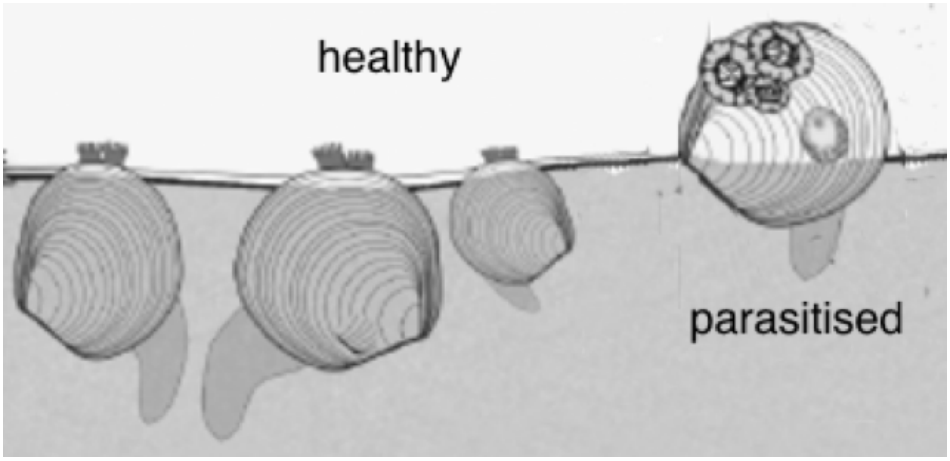


A schematic of how evolution works, with the example of body size. The blue line shows the assumption that fecundity (the number of offspring per time unit) increases with size; the red curve shows that longevity decreases with size. The black curve is the product of the two, giving the life-time reproductive success. (Each curve is scaled to have the same maximal value.)

(For those of you with an economic background, this is the evolutionary equivalent of the Laffer curve, which is a representation of the relationship between rates of taxation and the resulting levels of government revenue. It illustrates the (disputed) idea that no tax revenue will be raised at the extreme tax rates of 0% and 100%, so that there must be a rate between 0% and 100% which maximizes government taxation revenue. The Laffer curve is typically represented as a graph which starts at 0% tax with zero revenue, rises to a maximum rate of revenue at an intermediate rate of taxation, and then falls again to zero revenue at a 100% tax rate.)

Try to find the optimal size analytically. Assume that fecundity m increases with size S according to $m(S) = FS - H$, and the longevity decreases with size according to $L_0 e^{-dS}$, with L_0 the longevity rate with no predation. How does the optimal size change if mortality rate increases more strongly with size (that is, if d increases)?

This idea is fundamental to understand the manipulation of behaviour by parasites. Let me illustrate it again with yet another trematode, *Curtuteria australis*. As you will by now know, the first intermediate host of this trematode is a snail. The second one is a cockle. Transmission to the final host, an oystercatcher, occurs when the bird eats an infected cockle. So far, the system seems analogous to *Microphallus papillorobustus*; since transmission requires that the cockle is eaten, you might expect that the parasite manipulates the cockle to make it easier to catch. Indeed, this is what we observe.



Left side: Healthy cockles bury into the sand to protect themselves against predation. Right side: Infected cockles cannot bury into the sand, so they are much easier prey for oystercatchers.

The cockles in this system occur in the intertidal zone. During the ebb, the cockles usually bury into the sand and are difficult to catch by the oystercatchers. However, the metacercariae of the parasite encyst in the foot of the cockle, which it uses to dig into the sand; they destroy the foot muscle, and stunt its growth. Infected cockles therefore lose their ability to bury; they therefore lie at the surface and are easy prey to oystercatchers. While this sounds like a simple story of manipulation, things get a bit complicated, if we look at some details.

First, some parasites are again freeloaders. The metacercariae are distributed along the foot. Only the ones at the tip impede the ability to bury, but all of them are transmitted to the oystercatcher, for the bird eats the whole cockle. Thus the parasites at the base of the foot profit from the manipulation by the ones at the tip.

Second, cockles are eaten by other predators, but in these, the parasite cannot complete its life-cycle. A predatory snail, for example, eats cockles, and the manipulated cockles are also easier prey for this predator. Since the parasites eaten by a snail die, manipulation leads to a cost. Like the oystercatcher, the snail eats the whole cockle, so manipulation is equally costly for the manipulator and the freeloader. Another predator is a fish, and this fish only takes the tasty parts of the cockle: the tip of the foot that is protruding from the shell. The fish thus takes the manipulating parasites, but not the freeloaders. Since the parasite cannot complete its life-cycle in the fish, this predation imposes a large cost of manipulation: while only 2.5% of the parasites

end up in the 'correct host', 17.1% are eaten by the fish. But this cost is carried only my the manipulators [14].

The question is thus: under what circumstances does it pay to manipulate the cockle, even if manipulation increases the chance of getting to the wrong host? Let's model a simplified version of this situation, where we have birds as the correct host and consider only fish as the wrong host. We again assume that N parasites infect the cockle and that the probability that at least one of them is a manipulator is f. If there is no manipulator in the cockle, the probability that the parasite is transmitted to either host (that is, that the cockle is eaten) is T, and the presence of at least one manipulator increases the probability that cockles are eaten to $T + \epsilon(1 - T)$. If the cockle is eaten, a manipulator may end up in a bird or a fish; let's call the probability that it ends up in a bird (that is, in the correct host) h. A free-loader can only end up in a bird, for the fish only eats the tip of the foot. Thus the transmission of a manipulator is

$$(T + \epsilon(1 - T))h$$

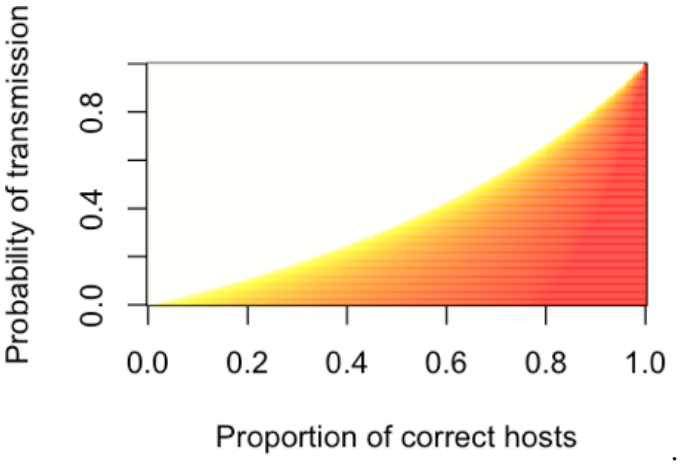
and that of a freeloader is

$$f(T + \epsilon(1 - T)) + (1 - f)T = Tf\epsilon(1 - T)$$

We can then find the condition where a manipulate has greater success than a free-loader as

$$f < \frac{h(T + \epsilon(1 - T)) - T}{\epsilon * (1 - T)}$$

Thus, the higher the proportion of the predators that are correct hosts and the lower the baseline transmission (with no manipulation), the more easily manipulation can invade.

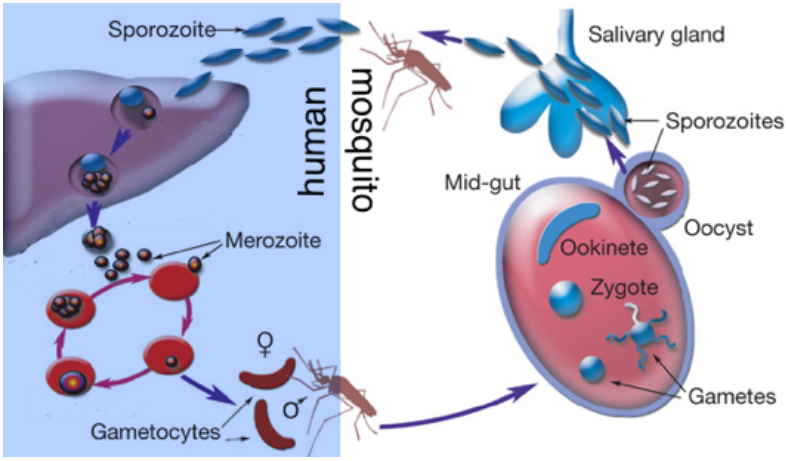


Now, do not assume that either of the two models I just formulated describe the details of the real situation. They are both crude caricatures of reality. I presented them to give the main message: that even in the absence of kin selection there are situations that make it profitable for parasites to manipulate their host despite often sacrificing themselves for the profit of freeloaders.

[14] Mouritsen K.N. & R. Poulin. 2003 Parasite-induced trophic facilitation exploited by a non-host predator: a manipulator's nightmare. International Journal of Parasitology 33:1043–1050.

Manipulation by malaria parasites

We can review and expand on several of these concepts by studying malaria. There are more than 100 species of *Plasmodium*. Five of them cause malaria in humans; the others infect a range of vertebrates including lizards, birds, mice and primates. They all have a life cycle similar to what is shown in the figure below. After infection and a first round of replication in the liver, parasites infect blood cells, replicate within them, kill them and go on to infect other blood cells. This repeated round of replication leads to a very high parasite load and can lead to considerable anaemia. At some point, parasites no longer replicate, but produce a stage that can be transmitted to the mosquito. In the mosquito, there is again a period of replication before the parasite produces the stage that can be transmitted to humans. What you should remember about this life cycle, for our discussion, is that there are stages for replication in the vertebrate (merozoites that go on to replicate inside the blood cells) and the mosquito (oocysts), and that there are other, distinct stages for transmission to the next host (gametocytes in humans and sporozoites in mosquitoes). Neither merozoites nor oocysts can be transmitted.

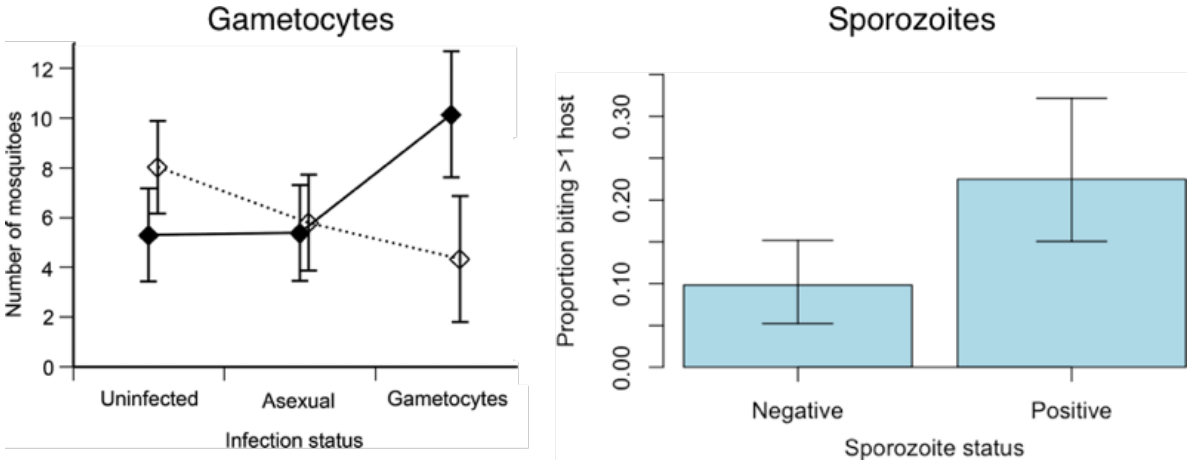


Life-cycle of the human malaria parasite *Plasmodium falciparum*

So, how would we expect the different stages to manipulate their host to increase the parasite's transmission? Let's start with the gametocytes. What limits transmission is the number of mosquitoes to which the gametocytes can be transmitted. Could the parasite increase this number? Mosquitoes are attracted to their host by its odour. For example, the main vector of malaria in Africa, *Anopheles gambiae*, is attracted by the odour of smelly feet. In fact, this odour is produced by bacteria that are related to those in Limburger cheese. You can even bait traps with Limburger cheese to attract the mosquitoes (and to kill them, if an insecticide is added to the trap). You might therefore imagine that the gametocytes (but not other stages in the vertebrate) change the odour of their host to make them more smelly (that is, attractive). And indeed, that is what happens [15]. If hungry mosquitoes are put into a situation where they can fly towards (and would bite, if they were not prevented from doing so) either an uninfected child, one with the stages of the parasite that are replicating in the blood (but without gametocytes), or one with gametocytes (in addition to the replicating stages), about twice as many mosquitoes, on average, are attracted to the gametocytic child as to either of the other two. Perhaps most remarkably, if the same children are tested a second time a few weeks later, once their parasite has been

[15] Lacroix, L. 2005. Malaria infection increases attractiveness of humans to mosquitoes. PLoS Biology 3:e298

cleared by an antimalarial drug, the mosquitoes show no preference at all. This suggests that it is indeed the infection by gametocytes that makes children more attractive to mosquitoes. One can go a step further in the laboratory with a malaria species that infects mice, and show that the mechanism by which gametocytes attract mosquitoes is indeed that the parasites change the odour of the mice [16]. It is possible to collect the odours of uninfected and infected mice, present these to mosquitoes, and test whether mosquitoes prefer one or the other. If the odours are collected at times when the parasites have not yet produced gametocytes, the mosquitoes prefer the odour of the uninfected mice; when the infection is older and the parasites have started to produce gametocytes, the mosquitoes prefer the odour of the infected ones. Furthermore, several compounds, for example hexanoic acid and 3-methyl butanoic acid, are enhanced in the odour of gametocytic, but not pre-gametocytic mice. If these odours are added to that of uninfected mice, mosquitoes prefer the combined odour.



Manipulation of mosquitoes by malaria parasites in natural situations. The left-hand panel shows that children harbouring gametocytes attract more mosquitoes that other children, but that these children are no longer most attractive once they have cleared their infection. The right-hand panel shows that mosquitoes with sporozoites are more than twice as likely to bite several people than those without sporozoites.

Let's move to the other side of the cycle, to the sporozoites. These are transmitted each time a mosquito bites. Or more precisely, they are transmitted each time a mosquito injects a bit of saliva into its host, which occurs at the beginning of a bite. Therefore, transmission is limited by the frequency of biting (rather than, say, the duration of each bite). Malaria should therefore try to increase the mosquito's biting rate. Again, this is what it does. It is such an important aspect of manipulation for the parasite that it has several mechanisms to achieve it. To understand them, you need to understand a bit of mosquito behaviour. First, if a mosquito is hungry and tries to bite you, you can scare it away, but it will then come back for a second try. You swat at it again, it comes back, you scare it, and so on, until either you give up and let it bite or the mosquito gives up. Second, if a mosquito is fully fed, it will fly away and not feed again before it has laid its eggs a few days later. If, however, it is not fully fed, it may come back to top up its blood-meal; the smaller its first blood-meal the more likely it will come back. Malaria sporozoites influence both of these behaviours [17] (though most of the physiological mechanisms are unknown) (i) If the mosquito is unfed, it takes longer before it gives up, if it is continuously prevented from getting a blood-meal. In other words, the mosquito is more motivated to bite. (ii) The parasite destroys

[16] De Moraes, C,M, et al. 2014. Malaria-induced changes in host odors enhance mosquito attraction. Proceedings of the National Academy of Sciences 111:11079-11084.

[17] Schwartz, A. & J.C. Koella. 2001. Trade-offs, conflicts of interest and manipulation in *Plasmodium*–mosquito interactions. Trends in Parasitology 17:189-194.

an enzyme in the saliva that acts as an anti-coagulant, which makes it more difficult for the mosquito to blood-feed. Therefore, in the limited time a biting mosquito has before it is swatted away, it takes up less blood and is therefore more likely to come back for more, if it is infected than if has has the normal anti-coagulant. (iii) In addition, at any given size of the blood-meal, the parasite makes it more likely that the mosquito returns for a top-up. (iv) This is even the case for fully fed mosquitoes; some infective mosquitoes keep on trying to bite, even if they are so full that they cannot take up any more blood. Each of these behaviours increases the frequency of biting and therefore, more importantly, the number of hosts that an infected mosquito bites and transmits the parasite to.

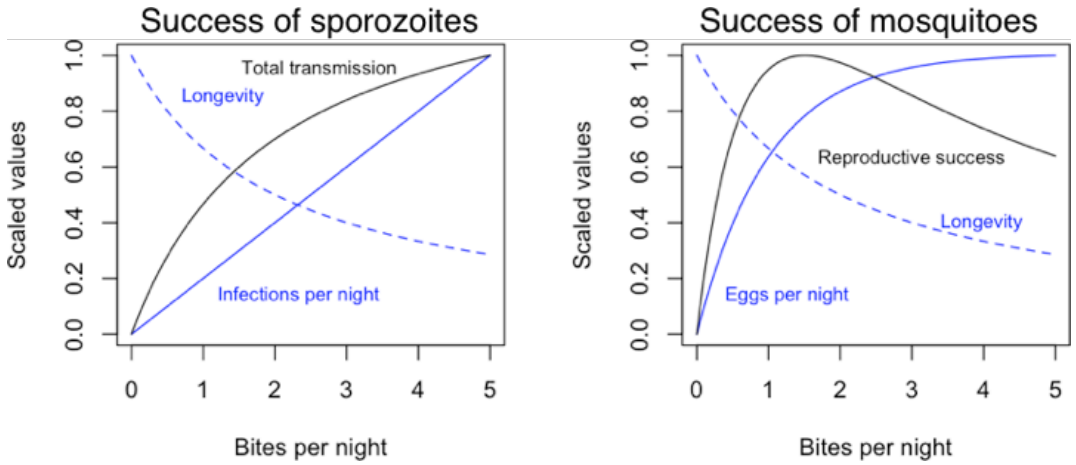
Finally, we consider the oocysts. You may remember that these cannot be transmitted; all they do for malaria is act as a replicator within which the transmissible sporozoites are produced. The only interest of the oocysts is therefore that the mosquito survives until their development is completed. Since biting is risky, this means that the oocysts should decrease the frequency at which mosquitoes bite. You will probably not be surprised that this is what they do. Whenever the behaviour of mosquitoes with oocysts has been compared to that of mosquitoes with sporozoites, the oocysts do the opposite of the sporozoites. They decrease the motivation to feed when they are not yet fed; they decrease the probability that mosquitoes return to top up their blood-meal.

The number of ways that malaria appears to manipulate mosquitoes is remarkable. But is the manipulation by, for example, the sporozoites really an advantage to the parasite? You might doubt that it is, because biting is one of the most risky behaviours of mosquitoes. The more they bite, the more likely they are to be killed by the person they are biting. The more blood they have have taken up, the more likely they are to be eaten by their predators, for fully fed mosquitoes offer more nutrition, are far more conspicuous and react far more slowly than uninfected ones. Jumping spiders, for example, prefer blood-fed over unfed mosquitoes; even if a spider is in the process of eating an unfed mosquito, it will let go of it and attack a fully fed mosquito that happens to approach. So the question (as above) is: does the advantage of manipulation by the sporozoites - transmission to more hosts - really outweigh its disadvantage - a shorter life-span?

We will again try to answer the question with a mathematical model that shows how advantages and disadvantages are balanced. The total transmission of a parasite (once it is a sporozoite) is the number of bites per night multiplied by the number of nights the mosquito lives. On the one hand, we know that every bite has a certain probability of infecting the host. (The probability depends on many factors, for example the sporozoite load, but we assume here that for a given mosquito it is constant.) Therefore, the number of infections per night (T) is proportional to the number of bites (B): $T = \gamma B$, where $$\gamma$$ is a proportionality factor. On the other hand, we can assume that every bite brings with it a certain risk that the mosquito is killed: $\mu = mu_0 + \delta B$. (The expected longevity is the inverse of the mortality rate, so $L = 1/(mu_0 + \delta B)$. (This relationship between mortality rate and average longevity is a general relationship that you should remember.) The left-hand panel below shows both of these curves and the total transmission success, which is the product of the two. It shows that the best strategy for sporozoites is to manipulate the mosquito as much as it can, even if manipulating the mosquito shortens its life-span. (And indeed, manipulation does shorten the life-span, at least in one experiment in Tanzania [18], in which about 12% of the mosquitoes carried sporozoites early in the evening (before the mosquitoes bite), but only about 8% of them did the next morning after

[18] Anderson R.A., B.G. Knols & J.C. Koella. 2000. *Plasmodium falciparum* sporozoites increase feeding-associated mortality of their mosquito hosts *Anopheles gambiae s.l.* Parasitology 120:329-33.

a night of biting. The difference is most likely due to biting-induced mortality, which is higher in the infected mosquitoes.)



Simple models describing the transmission success of sporozoites (left-hand panel) and the reproductive success of mosquitoes (right-hand panel). The solid blue curves show the number of infectious bites per night for the parasite or the number of eggs that can be laid per gonotrophic cycle for the mosquito. The dashed blue line shows the expected longevity. The solid black line shows the total success. The values on each curve are divided by their maximal values, so that the curves can be shown in the same figure.

You may still object that the biting rate that is optimal for the parasite does not reflect manipulation; the same rate could also be what the infected mosquito wants. We therefore go through the same exercise, but consider the mosquito's evolutionary fitness, which can be approximated by the total number of eggs that it can lay. Now, the longevity of the mosquito is of course the same, whether we are considering the success of the parasite or the mosquito. What differs is the function for the rate of success. As the number of eggs is determined by the amount of blood, increased biting will not increase fecundity if the mosquito is already full and cannot take up no more blood. The relationship between fecundity and number of bites therefore flattens off to some maximal value. The consequence of this, as you can see in the right-hand plot above, is that the success of the mosquito is maximised at an intermediate biting rate. Thus, the parasite's optimal biting rate is higher than the mosquito's; it should try to manipulate the mosquito to bite more frequently than the mosquito would want. We can say that the manipulation we observe is indeed to the benefit of the parasite.

The question about the optimal manipulation by malaria is essentially a question about the evolution of virulence, which we will discuss in the next chapter. But let's already think about some aspects here. We saw that sporozoites should try to increase the mosquito's biting rate even though it increases the rate of the mosquito's mortality by doing so. Now, let's say we're studying manipulation of mosquitoes with long and with short life-spans (for example, different species, or a single species in populations with more or less predation.) Would you expect manipulation to be higher, the same, or lower in the species with a longer life?

Manipulation and epidemiology

Since malaria manipulates mosquitoes to increase its transmission, you can imagine that they thereby make the epidemiological situation worse.

As we will see later, a central concept of epidemiology is the *basic reproductive number* (abbreviated as R_0), which describes the number of secondary infections resulting from a single infected individual in an otherwise susceptible population. Many of our diseases have an R_0 that is less than about 20: the flu and ebola about 2, polio about 5, measles about 15. Smallpox had an R_0 of about 4. Now, quite generally, the larger R_0, the harder it is to control the epidemic. We have therefore been able to eradicate smallpox and are on the verge of eradicating polio, we can control ebola epidemics (though with considerable effort), we can vaccinate enough people to prevent large epidemics of measles. Compare these numbers with the R_0 of malaria, which can reach about 1000 in the most endemic areas. That means that a single infected person will end up infecting 1000 others! It is this very intense transmission that makes malaria so difficult to control. And a substantial part of this intense transmission is due to the many ways the parasite can manipulate the mosquitoes. Gametocytes increase transmission to mosquitoes, oocysts increase longevity of mosquitoes, sporozoites increase transmission to humans. We don't have good estimates of the impact of these manipulations on R_0, but if they are each on the order of a 2-fold change, R_0 would be close to 10 times lower were there no manipulation. Malaria would still be difficult to eliminate, but a lot easier to control.

Take-home points

- Many parasites manipulate the behaviour of their hosts to increase their transmission.
- Many descriptions of manipulation are over-simplications. To understand why and how much parasites manipulate their hosts, we need detailed analyses of costs and benefits.
- Manipulation can have an important impact on our health.