The impact of *Toxoplasma gondii* on the mammalian genome

Urs B Müller[1] and Jonathan C Howard[1,2,3]

Nobody doubts that infections have imposed specialisations on the mammalian genome. However sufficient information is usually missing to attribute a specific genomic modification to pressure from a specific pathogen. Recent studies on mechanisms of mammalian resistance against the ubiquitous protozoan parasite, *Toxoplasma gondii*, have shown that the small rodents presumed to be largely responsible for transmission of the parasite to its definitive host, the domestic cat, possess distinctive recognition proteins, and interferon-inducible effector proteins (IRG proteins) that limit the potential virulence of the parasite. The phylogenetic association of the recognition proteins, TLR11 and TLR12, with *T. gondii* resistance is weak, but there is evidence for reciprocal polymorphism between parasite virulence proteins and host IRG proteins that strongly suggests current or recent coevolution.

Addresses
[1] Institute for Genetics, University of Cologne, Zülpicher Strasse 47a, 50674 Cologne, Germany
[2] Instituto Gulbenkian de Ciência, Rua da Quinta Grande 6, 2780-156 Oeiras, Portugal
[3] Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, 50829 Cologne, Germany

Corresponding author: Howard, Jonathan C (j.howard@uni-koeln.de)

Infection modifies genomes

The mammalian genome has clearly been influenced by infection. The extraordinary genomic complexity of the rearranging receptors of lymphocytes and the complex array of immune functions assembled in the mammalian MHC are testimony to millions of years of pathogen pressure.

Less straightforward is to document where and how specific pathogens have triggered specific genomic effects. Recent fatal pandemics have left their marks on the human genome, for example in the shape of a number of more or less dysgenic alleles of α-globin and β-globin for malaria, witnessing the urgency and intensity of selection by novel pathogens. In mice the superantigenic ORF proteins of endogenous mammary tumor viruses appear to have taken a toll of T cell receptor Vβ families [1] as the selective priorities for the mouse seem to have favoured a sub-optimal T cell repertoire over the risk of inflammatory death.

The strongest evidence for a definite recent causal relationship between specific features of pathogen and host genomes is reciprocal polymorphism with an experimentally demonstrable causal chain. Apart from the classic examples noted above, this level of analysis has only occasionally been achieved, and most notably in plant disease resistance [2••]. When experimental data has been substantiated by ecological evidence, one may fairly describe such a scenario as co-evolution.

The evolutionarily significant host (ESH)

The pattern of host–pathogen co-evolution depends on the extent to which host resistance reduces pathogen transmission. Fast-evolving pathogens counter this cost by rapid evasive evolution. These familiar 'Red Queen'-like processes can result in polymorphic variation in host and pathogen as each attempts to sidestep the other. *Toxoplasma gondii* (*T. gondii*) is an extremely promiscuous pathogen, generating recombinational diversity through gametogenesis in all species of true cats, and all warm-blooded animals are potentially intermediate hosts (see Figure 1). Evolutionary significance of hosts for *T. gondii* is therefore not yes or no, but a quantitative parameter. Some species are certainly in this sense important hosts for transmission of the pathogen, others probably not, a distinction applying equally to definitive and intermediate hosts. In a comparison between important and less important hosts we might identify genomic signatures of the immune resistance machinery that reflect selective pressure from the parasite on the ESH.

The evolution of *T. gondii* will be driven in the foreseeable future by its relationship with the domestic cat as definitive host, but the absolute dominance of the domestic cat is recent and it is unknown whether any genomic coadaptation has already occurred. The limited genotypic diversity of *T. gondii* in the Old World compared with S. America may reflect an ancient S. American origin for the species [3] although there are arguments against this view [4]. In any case, the original genetic diversity of Old World *T. gondii* may have been

Figure 1

Current Opinion in Microbiology
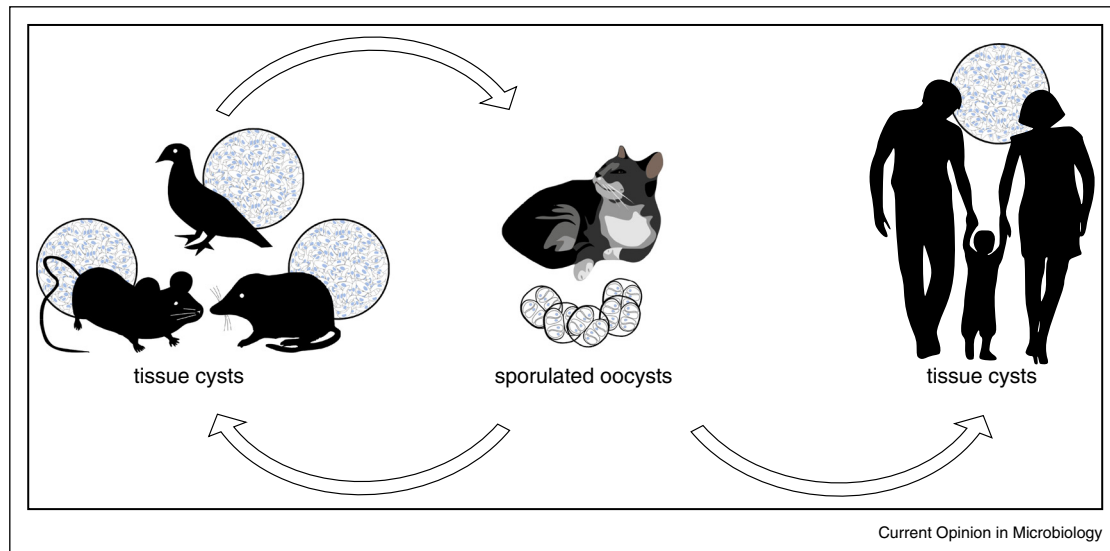
The *Toxoplasma gondii* life cycle, highlighting the distinction between evolutionarily relevant hosts and irrelevant hosts. The infected domestic cat excretes oocysts into the environment with the feces where they are inadvertently ingested by foraging animals. Humans may be infected via contact with cat feces in the domestic environment and in other ways. After a brief proliferative phase (tachyzoite) immunity may develop and restricts further growth. In this case the parasite undergoes a phase change to a slowly-replicating form (bradyzoite), building cysts in brain and muscle that persist for the life of the intermediate host. If the intermediate host is in the food-chain of domestic cats, as common birds and small mammals are, then it is relevant for transmission and of evolutionary relevance to the parasite. Infected hosts outside the food chain of the domestic cat, like humans, are irrelevant for transmission. The parasite must stimulate an effective immunity shortly after infection to ensure avirulence; failure to do so allows uncontrolled proliferation and the early death of the host. Virulence is clearly costly for the host and in mice and probably other small rodents. The highly polymorphic IRG system of IFNγ-inducible GTPases provides resistance against *T. gondii* strains of difference virulence without achieving sterile immunity. Presumably the selective advantage of the high virulence for mice, seen in some strains of *T. gondii*, reflects pressure on the parasite from other evolutionarily relevant hosts that can indeed achieve sterile immunity during primary infection. So far, high virulence has always been attributed to the ROP5/ROP18 complex of virulence proteins, known to target IRG proteins (see Figure 2), so it is likely IRG proteins are also required for the induction of sterile immunity in such hosts.

larger and the recent expansion of the domestic cat, an Old World species until the sixteenth century, may have favoured a specific subset of pre-adapted genotypes.

The identification of dominant ESH species as intermediate hosts is more complex, but mammal or bird species that are rare or inaccessible as prey for domestic cats must be low down on the hierarchy, while species that are abundant and accessible are high up. Humans, on the other hand, while abundant and globally infected by *T. gondii* at a rate over 1% per year of age [5[•]], are inaccessible as prey for domestic cats and can be eliminated as an ESH. The parasite is completely uninterested in defeating, or being defeated by, human immunity. In the event, while human immunity is normally sufficient to reduce morbidity from *T. gondii* infection to very low levels, the parasite's exceptional ability to use host immunity in general as a trigger for bradyzoite conversion means that infected humans do carry cysts and so far no immunity sufficient for parasite elimination has yet been recorded in man. What we may fairly say is that no components of human immunity

seem to be specifically dedicated to resistance against *T. gondii*. The human genome thus seems to provide a reasonably reliable negative control.

What about the strong ESH candidates? Cat and mouse are global species and sympatric. Furthermore, foraging mice should have a significant chance of ingesting oocysts spread in cat feces. Infection rates in urban *Mus musculus* above 50% have been reported in the UK [6], but much lower rates (0–5%) are more general [7–10]. In US studies, infection of wild *M. musculus* is reported to be in the low range (0–3% [11,12]) and values for the US native mouse, *Peromyscus*, are similar [13]. Since unconfined domestic cats defecate and hunt in the natural environment adjacent to their homes, rather than at home, the ecology of *Apodemus* and other local wild-life may be more relevant to the evolution of modern *T. gondii* in Europe than that of *M. musculus*. Significant infection rates have been reported in the European field mouse, *Apodemus* [14,15] as well as in voles and shrews [8,16], abundant Eurasian small mammals often found near human habitation but scarcely overlapping in range with the domestic mouse. Likewise, domestic cats regrettably catch the

common wild songbirds that live with us, as well as unloved but abundant urban feral rock pigeons. These may also be important ESH species, but it is certain that a significant proportion of *T. gondii* pass through *M. musculus* during the generational cycle and the mouse is certainly the best-known candidate for a species with significant ESH credentials.

Immune mechanisms against Toxoplasma in mice and humans

Two striking differences between mouse and man have been highlighted, a recognition mechanism and an effector mechanism. In mice, innate recognition of *T. gondii* infection depends on two members of the TLR (Toll-like receptor) family, TLR11 and TLR12, probably forming a heterodimer [17], and the trigger was identified as *T. gondii*-profilin [18]. Both TLR proteins are absent in human [18,19]. Without them, mice are susceptible to normally avirulent *T. gondii* strains. Secondly, members of a family of 10–20 interferon-γ-inducible GTPases, the IRG proteins [20], assemble on and disrupt the parasitophorous vacuole membrane [21]. IRG proteins are essential for mouse survival from normally avirulent *T. gondii* infection [22,23]. Humans express only one non-inducible IRG fragment, IRGM, of uncertain function. A further family of interferon-inducible GTPases, the 65 kDa guanylate binding proteins (GBP), is present in both species. In the mouse GBPs assemble on a proportion of IRG-loaded parasitophorous vacuoles [24] and contribute to the strength of IFNγ-inducible resistance [25–27]. In the human, GBPs do not assemble on parasitophorous vacuoles although a resistance function distant from the vacuole has been proposed [28•].

Much of the immune machinery involved in resistance against *T. gondii* is, however, common to man and mouse, forming the general innate-adaptive response axis: macrophages, dendritic cells, IL-12, IFNγ, CD4 and CD8 T cells, CD40, the MHC, as well as NO and active oxygen radicals are all implicated in resistance against *T. gondii* [29,30]. It was shown recently that polyubiquitin is deposited on the vacuolar membrane in both mouse and human cells [31•,32•,33•]. Human resistance against *T. gondii* is remarkably effective despite the absence of TLR11/12 and IRG proteins. Tryptophan depletion by the catabolic action of an IFNγ-inducible indoleamine dioxygenase has been implicated in restricting *T. gondii* growth in human cells [34], but this has not been generalizable over cell types and culture conditions [35]. The human NLRP1 inflammasome has also been implicated as an initiator of some cell-autonomous immunity in human macrophages in the absence of IFNγ [36], but the effector mechanism is unknown. Human TLR5 has recently been shown to be triggered by *T. gondii*-profilin [37], arguably replacing TLR11/12. Perhaps immunity of humans against *T. gondii* is the sum of small effects. Certainly the human mechanism in its entirety does not exist in mice since loss of IRG or TLR11/12 proteins is fatal. Thus we have a clear dichotomy: mice have the essential TLR11/12 and IRG mechanisms but not the human mechanisms, while humans have their mechanisms, whatever they may be, but not the TLR11/12 and IRG mechanism.

What do these differences mean?

The known specializations of the mouse accompany its ESH status [38,39•]. It was further recently found that IL-12 production in mice is triggered by live parasite invasion, in human by phagocytosis [40] and the authors state: 'possibly reflecting a direct involvement of rodents and not humans in the parasite life cycle.' However while mouse is an ESH and human not, no causal connection has been offered. Gazzinelli and colleagues [39] tried to strengthen the link, both for TLR11/12 and for IRG proteins, by looking at a wider range of species, but no convincing correlation emerged. IRG genes were certainly most abundant in small rodents, but nearly absent in rabbits. Horses, an unlikely prey for small cats, have no recorded IRG genes but their relative abundance in elephants and manatees stretches the correlative argument too thin. The same problem afflicts the TLR11/12 distribution, present in rodents and lagomorphs, but also in horses, rhinos, elephants and manatees. Absent or pseudogenised in humans, orcas, dogs and cats, the expression of TLR11/12 seems to associate inversely with carnivory, but this correlation too is destroyed by their absence also from the obligate herbivore, the giant panda.

Reciprocal polymorphism strengthens the argument

The correlative argument contains no causal link. If, however, host and pathogen show reciprocal polymorphism in virulence and resistance, it would suggest that the system is under selection. In the TLR11/12 recognition/response mechanism against *T. gondii*-profilin, no relevant polymorphism was found correlating with infection status in *Apodemus* [41•]. However in the IRG system in mice, there is functional reciprocal polymorphism with *T. gondii* virulence proteins [42]. Eurasian *T. gondii* strains designated type I are virulent for laboratory mice, for example, C57BL/6. Differential virulence of *T. gondii* strains is due to allelic variation in two homologous, polymorphic secreted proteins, a kinase (ROP18) and a pseudokinase (ROP5). Together, these phosphorylate two conserved threonines on effector IRG proteins and inactivate them [43]. Type I strains are, however, resisted by a wild-derived mouse strain, CIM, from South India [42]. In crosses between CIM and C57BL/6, all the resistance maps to a highly polymorphic IRG gene cluster on chromosome 11. The polymorphic surface of the pseudokinase ROP5 binds the nucleotide-binding domain of Irga6 adjacent to the target threonines [42,44]. This surface region, on the homologous Irgb2-b1 protein encoded on chromosome 11, is the same region that shows evidence

Figure 2
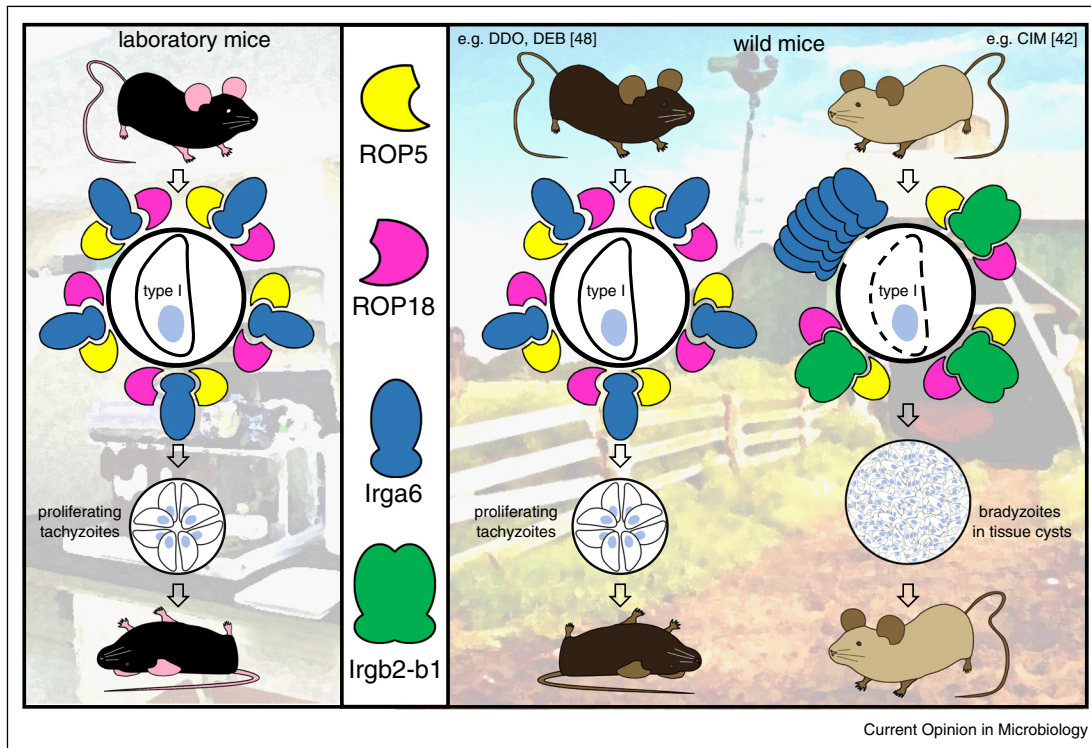
Susceptibility and resistance of mouse genotypes against type I virulent strains of *T. gondii*. A typical laboratory inbred strain such as C57BL/6 (left panel) expresses effector IRG proteins such as Irga6 that are phosphorylated and inactivated by a kinase complex of ROP5 and ROP18 secreted by the parasite. Immunity against *T. gondii* fails and the mice die within a few days of infection. Such susceptible IRG alleles are segregating in wild mouse populations with other IRG alleles that confer resistance to the virulent kinase complex (right panel). In particular, the highly polymorphic 'tandem' IRG protein, Irgb2-b1, of such resistant strains (e.g. CIM) acts as a decoy, diverting the active kinase complex from effector IRG proteins and allowing adequate immunity to develop to control the tachyzoite growth phase. In a mouse with such a genotype, 'virulent' *T. gondii* strains become avirulent, form cysts, and can be transmitted. Other wild strains (such as DDO from Denmark and DEB from Spain) carry susceptible b2b1 haplotypes [48] and will be as vulnerable as C57BL/6. We hypothesize that ROP kinases highly virulent for mice arise under pressure from evolutionarily relevant intermediate hosts that express IRG proteins capable of yielding sterile immunity to a primary infection. So far, sterile immunity is unknown in mice: we presently view the origin of the highly resistant Irgb2-b1 alleles of mice as a response to pressure from highly virulent *T. gondii* strains.

of recent directional selection. Irgb2-b1 from the CIM mouse transfected into C57BL/6 cells blocks phosphorylation of Irga6 by a virulent type I strain of *T. gondii*.

In this analysis, the argument favouring a causal chain from virulence to resistance is complete. The polymorphic variation of Irgb2-b1 'matches' the polymorphic variation of parasite ROP5 and the results have biological meaning. Type I strains that are virulent in mice carrying the laboratory mouse allele of Irgb2-b1 kill their host within a few days and thereby essentially eliminate the chance of their own transmission. In mice carrying the CIM allele of Irgb2-b1, however, both parasite and host profit; the parasite can encyst in a resistant host, while the host lives out a normal life (Figure 2).

These results leave us with a number of questions. It has recently been shown that much of the virulence of S. American *T. gondii* strains for laboratory mice is also

due to alleles expressed at ROP5 [45**]. Can we conclude that the allelic variation in ROP5 across multiple parasite strains globally is all directed at allelic variants of IRG proteins? Or are different ROP5 alleles directed at entirely different target proteins relevant to different ESH species? The house mouse is a Eurasian species, yet most of the polymorphic variation in ROP5 is found among the enormous diversity of S. American strains [35]. Which species are ESHs in S. America and what if any IRG proteins do they have? Since the resistant allele of Irgb2-b1 is advantageous to both host and parasite at least in Eurasia, why is it not fixed? What selection pressure has led to the evolution of the susceptible Irgb2-b1 allele of the laboratory mouse strains and to its greatly reduced expression level (unpublished results Lilue & Müller)?

Just as the polymorphic virulence factors of *T. gondii* may have different molecular targets in different ESH species, so the IRG resistance system is certainly not directed

exclusively at *T. gondii*. Polymorphic variants of the IRG system found among laboratory mouse strains also regulate resistance to *Chlamydia trachomatis* [46] and *Chlamydia psittaci* [47], while IRG proteins are also essential for resistance of mouse cells against the microsporidian fungus, *Encephalitozoon cuniculi*, although differential resistance has not been shown for IRG alleles [48]. Both Chlamydiales and Microsporidia are ubiquitous and abundant pathogen classes and may well be more important for the evolutionary dynamics of the IRG system than *Toxoplasma*.

The struggle for avirulence

The strategy of *T. gondii* as a parasite is based on a quest for avirulence, a capacity to attenuate but not to destroy the immune resistance of the host, thus securing the permanent residence required to await transmission. How the parasite achieves this ideal state in thousands of potential hosts, with strikingly different immune systems is the major unknown in *T. gondii* biology. This power is analogous to the ability of the adaptive immune system of vertebrates to resist thousands of different pathogens. The adaptive immune system shows little co-adaptation at a genomic level to different pathogens; it is a general anti-pathogen machine. Likewise, *T. gondii* has a general anti-host machine, not perfect, but able to titrate host immunities of many different kinds against the self-destructive potential of its own replicative powers. Armed with this instrument, whatever it consists of, it is presumably irrelevant whether a specific host species is an ESH or not. Polymorphic variation in ROP5 and ROP18 is essential in mice, that use the IRG system, but irrelevant in humans, that do not [35]. Presumably the polymorphism and regulation of other genetic systems are essential against different immune resistance mechanisms favoured by other host species. *T. gondii* sometimes fails to achieve its goal of avirulence in geographically incoherent infections; some strains of S. American *T. gondii* are highly virulent in humans [49], a non-native species, and many S. American strains are highly virulent for laboratory mice, which represent W. European *M. m. domesticus* >90% genetically [50]. Likewise, the type I Eurasian strains relatively frequent in the far East are highly virulent for laboratory mouse strains but avirulent for *M. m. castaneus* strains from the East Asian region. These instances hint at further co-evolution between *T. gondii* and its intermediate hosts.

Conclusion

For the moment the proven relationship between polymorphic virulence alleles of *T. gondii* and the proven resistance alleles of the IRG system of mice presents the strongest evidence that this host pathogen-pair are now or have recently been in a dynamic co-evolutionary relationship of sufficient intensity to contribute to genome modification through allelic diversification by both partners. The weak correlation in species distribution of

the TLR11/12 pair possibly suggests that this recognition system also helps or has recently helped several mammals in immunity against *T. gondii*, but does not tell us that selection by this organism has brought it into existence, any more than that it is likely that H-2L^d, for example, which is known to present several *T. gondii* peptides to T cells [51,52] owes its existence to the parasite, and there is no evidence yet in either case of a dynamic co-evolutionary process at work.

T. gondii has evolved a complex orchestra of actions that play on vertebrate pathogen resistance machinery and except in the case of the IRG system there is little reason to believe that host resistance machinery is anything other than beneficial to both host and parasite in enabling the avirulent state and encystment. The polymorphism of Irgb2-b1 and its intimate association with the virulence polymorphism of ROP5 and ROP18 raises the question, what selection generates type I virulence in *T. gondii* strains where *M. musculus* is an ESH? Avirulent types II and III strains can also encyst in the highly resistant CIM mice so there is no 'need' for extra virulence. Arguably, the type I virulent strains are preferentially adapted to another important ESH species, perhaps a rat, whose IRG system is capable of enforcing sterile immunity on strains lacking the virulent alleles of ROP5 and ROP18. The Irgb2-b1 allele of mice would then be accounted for as an essential adaptation for mice living within the range of such virulent strains, perhaps typically in the Far East. Polymorphic variation in the IRG system is probably also driven by other parasites as well, for example *Chlamydia* or Microsporidia. Allelic frequencies will depend on the ratio of the intensity of selection pressures from the parasites.

Much work will be required and at many different analytical levels, genetic, biochemical, structural, ecological and immunological, to clarify these issues.

Acknowledgements

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- •• of outstanding interest

1. Pullen AM, Potts W, Wakeland EK, Kappler J, Marrack P: **Surprisingly uneven distribution of the T cell receptor V beta repertoire in wild mice**. *J Exp Med* 1990, **171**:49-62.

2. Ellis JG: **Integrated decoys and effector traps: how to catch a**
•• **plant pathogen**. *BMC Biol* 2016, **14**:13.
An exceptional review of well-accredited plant host–pathogen interactions with both ecological and molecular support for evolutionarily important interactions.

3. Lehmann T, Marcet PL, Graham DH, Dahl ER, Dubey JP: **Globalization and the population structure of *Toxoplasma gondii***. *Proc Natl Acad Sci U S A* 2006, **103**:11423-11428.

4. Sibley LD, Ajioka JW: **Population structure of *Toxoplasma gondii*: clonal expansion driven by infrequent recombination and selective sweeps**. *Annu Rev Microbiol* 2008, **62**:329-351.

5. Wilking H, Thamm M, Stark K, Aebischer T, Seeber F: **Prevalence,**
• **incidence estimations, and risk factors of *Toxoplasma gondii* infection in Germany: a representative, cross-sectional, serological study**. *Sci Rep* 2016, **6**:22551.
Using well-curated German data, Seeber and colleagues are able to specify an infection rate for *T. gondii* in humans at 1% per year.

6. Murphy RG, Williams RH, Hughes JM, Hide G, Ford NJ, Oldbury DJ: **The urban house mouse (*Mus domesticus*) as a reservoir of infection for the human parasite *Toxoplasma gondii*: an unrecognised public health issue?** *Int J Environ Health Res* 2008, **18**:177-185.

7. Kijlstra A, Meerburg B, Cornelissen J, De Craeye S, Vereijken P, Jongert E: **The role of rodents and shrews in the transmission of *Toxoplasma gondii* to pigs**. *Vet Parasitol* 2008, **156**:183-190.

8. Meerburg BG, De Craeye S, Dierick K, Kijlstra A: ***Neospora caninum* and *Toxoplasma gondii* in brain tissue of feral rodents and insectivores caught on farms in the Netherlands**. *Vet Parasitol* 2012, **184**:317-320.

9. Muradian V, Ferreira LR, Lopes EG, Esmerini Pde O, Pena HF, Soares RM, Gennari SM: **A survey of *Neospora caninum* and *Toxoplasma gondii* infection in urban rodents from Brazil**. *J Parasitol* 2012, **98**:128-134.

10. Hurkova-Hofmannova L, Qablan MA, Jurankova J, Modry D, Pialek J: **A survey of *Toxoplasma gondii* and *Neospora caninum* infecting house mice from a hybrid zone**. *J Parasitol* 2014, **100**:139-141.

11. Smith KE, Zimmerman JJ, Patton S, Beran GW, Hill HT: **The epidemiology of toxoplasmosis on Iowa swine farms with an emphasis on the roles of free-living mammals**. *Vet Parasitol* 1992, **42**:199-211.

12. Smith DD, Frenkel JK: **Prevalence of antibodies to *Toxoplasma gondii* in wild mammals of Missouri and east central Kansas: biologic and ecologic considerations of transmission**. *J Wildl Dis* 1995, **31**:15-21.

13. Dabritz HA, Miller MA, Gardner IA, Packham AE, Atwill ER, Conrad PA: **Risk factors for *Toxoplasma gondii* infection in wild rodents from central coastal California and a review of *T. gondii* prevalence in rodents**. *J Parasitol* 2008, **94**:675-683.

14. Thomasson D, Wright EA, Hughes JM, Dodd NS, Cox AP, Boyce K, Gerwash O, Abushahma M, Lun ZR, Murphy RG *et al.*: **Prevalence and co-infection of *Toxoplasma gondii* and *Neospora caninum* in *Apodemus sylvaticus* in an area relatively free of cats**. *Parasitology* 2011, **138**:1117-1123.

15. Bajnok J, Boyce K, Rogan MT, Craig PS, Lun ZR, Hide G: **Prevalence of *Toxoplasma gondii* in localized populations of *Apodemus sylvaticus* is linked to population genotype not to population location**. *Parasitology* 2015, **142**:680-690.

16. Gotteland C, Chaval Y, Villena I, Galan M, Geers R, Aubert D, Poulle ML, Charbonnel N, Gilot-Fromont E: **Species or local environment, what determines the infection of rodents by *Toxoplasma gondii*?** *Parasitology* 2014, **141**:259-268.

17. Andrade WA, Souza Mdo C, Ramos-Martinez E, Nagpal K, Dutra MS, Melo MB, Bartholomeu DC, Ghosh S, Golenbock DT, Gazzinelli RT: **Combined action of nucleic acid-sensing Toll-like receptors and TLR11/TLR12 heterodimers imparts resistance to *Toxoplasma gondii* in mice**. *Cell Host Microbe* 2013, **13**:42-53.

18. Yarovinsky F, Zhang D, Andersen JF, Bannenberg GL, Serhan CN, Hayden MS, Hieny S, Sutterwala FS, Flavell RA, Ghosh S *et al.*:

19. **TLR11 activation of dendritic cells by a protozoan profilin-like protein**. *Science* 2005, **308**:1626-1629.

19. Roach JC, Glusman G, Rowen L, Kaur A, Purcell MK, Smith KD, Hood LE, Aderem A: **The evolution of vertebrate Toll-like receptors**. *Proc Natl Acad Sci U S A* 2005, **102**:9577-9582.

20. Bekpen C, Hunn JP, Rohde C, Parvanova I, Guethlein L, Dunn DM, Glowalla E, Leptin M, Howard JC: **The interferon-inducible p47 (IRG) GTPases in vertebrates: loss of the cell autonomous resistance mechanism in the human lineage**. *Genome Biol* 2005, **6**:R92.

21. Howard JC, Hunn JP, Steinfeldt T: **The IRG protein-based resistance mechanism in mice and its relation to virulence in *Toxoplasma gondii***. *Curr Opin Microbiol* 2011, **14**:414-421.

22. Taylor GA, Collazo CM, Yap GS, Nguyen K, Gregorio TA, Taylor LS, Eagleson B, Secrest L, Southon EA, Reid SW *et al.*: **Pathogen-specific loss of host resistance in mice lacking the IFN-gamma-inducible gene IGTP**. *Proc Natl Acad Sci U S A* 2000, **97**:751-755.

23. Collazo CM, Yap GS, Sempowski GD, Lusby KC, Tessarollo L, Woude GF, Sher A, Taylor GA: **Inactivation of LRG-47 and IRG-47 reveals a family of interferon gamma-inducible genes with essential, pathogen-specific roles in resistance to infection**. *J Exp Med* 2001, **194**:181-188.

24. Degrandi D, Konermann C, Beuter-Gunia C, Kresse A, Wurthner J, Kurig S, Beer S, Pfeffer K: **Extensive characterization of IFN-induced GTPases mGBP1 to mGBP10 involved in host defense**. *J Immunol* 2007, **179**:7729-7740.

25. Yamamoto M, Okuyama M, Ma JS, Kimura T, Kamiyama N, Saiga H, Ohshima J, Sasai M, Kayama H, Okamoto T *et al.*: **A cluster of interferon-gamma-inducible p65 GTPases plays a critical role in host defense against *Toxoplasma gondii***. *Immunity* 2012, **37**:302-313.

26. Degrandi D, Kravets E, Konermann C, Beuter-Gunia C, Klumpers V, Lahme S, Wischmann E, Mausberg AK, Beer-Hammer S, Pfeffer K: **Murine guanylate binding protein 2 (mGBP2) controls *Toxoplasma gondii* replication**. *Proc Natl Acad Sci U S A* 2013, **110**:294-299.

27. Selleck EM, Fentress SJ, Beatty WL, Degrandi D, Pfeffer K, Virgin HWt, Macmicking JD, Sibley LD: **Guanylate-binding protein 1 (Gbp1) contributes to cell-autonomous immunity against *Toxoplasma gondii***. *PLoS Pathog* 2013, **9**:e1003320.

28. Johnston AC, Piro A, Clough B, Siew M, Virreira Winter S, Coers J,
• Frickel EM: **Human GBP1 does not localise to pathogen vacuoles but restricts *Toxoplasma gondii***. *Cell Microbiol* 2016.
A slightly obscure but possibly interesting recent study hinting at novel properties for the interferon-inducible GBP protein family.

29. Yarovinsky F: **Innate immunity to *Toxoplasma gondii* infection**. *Nat Rev Immunol* 2014, **14**:109-121.

30. Pittman KJ, Knoll LJ: **Long-term relationships: the complicated interplay between the host and the developmental stages of *Toxoplasma gondii* during acute and chronic infections**. *Microbiol Mol Biol Rev* 2015, **79**:387-401.

31. Haldar AK, Foltz C, Finethy R, Piro AS, Feeley EM, Pilla-
• Moffett DM, Komatsu M, Frickel EM, Coers J: **Ubiquitin systems mark pathogen-containing vacuoles as targets for host defense by guanylate binding proteins**. *Proc Natl Acad Sci U S A* 2015, **112**:E5628-E5637.
These three recent papers have opened a new perspective on effector processes, implicating a ubiquitin-mediated autophagic step in control of *T. gondii* in both mouse and man. It will be interesting to see how this line of thinking develops.

32. Lee Y, Sasai M, Ma JS, Sakaguchi N, Ohshima J, Bando H,
• Saitoh T, Akira S, Yamamoto M: **p62 plays a specific role in interferon-gamma-induced presentation of a toxoplasma vacuolar antigen**. *Cell Rep* 2015, **13**:223-233.
See annotation to Ref. [31•].

33. Selleck EM, Orchard RC, Lassen KG, Beatty WL, Xavier RJ,
• Levine B, Virgin HW, Sibley LD: **a noncanonical autophagy pathway restricts *Toxoplasma gondii* growth in a**

strain-specific manner in IFN-gamma-activated human cells. *MBio* 2015, **6** e01157-01115.
See annotation to Ref. [31*].

34. Pfefferkorn ER: **Interferon gamma blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host cells to degrade tryptophan**. *Proc Natl Acad Sci U S A* 1984, **81**:908-912.

35. Niedelman W, Gold DA, Rosowski EE, Sprokholt JK, Lim D, Farid Arenas A, Melo MB, Spooner E, Yaffe MB, Saeij JP: **The rhoptry proteins ROP18 and ROP5 mediate *Toxoplasma gondii* evasion of the murine, but not the human, interferon-gamma response**. *PLoS Pathog* 2012, **8**:e1002784.

36. Witola WH, Mui E, Hargrave A, Liu S, Hypolite M, Montpetit A, Cavailles P, Bisanz C, Cesbron-Delauw MF, Fournie GJ *et al.*: **NALP1 influences susceptibility to human congenital toxoplasmosis, proinflammatory cytokine response, and fate of *Toxoplasma gondii*-infected monocytic cells**. *Infect Immun* 2011, **79**:756-766.

37. Salazar Gonzalez RM, Shehata H, O'Connell MJ, Yang Y, Moreno-Fernandez ME, Chougnet CA, Aliberti J: ***Toxoplasma gondii*-derived profilin triggers human toll-like receptor 5-dependent cytokine production**. *J Innate Immun* 2014, **6**:685-694.

38. Pifer R, Yarovinsky F: **Innate responses to *Toxoplasma gondii* in mice and humans**. *Trends Parasitol* 2011, **27**:388-393.

39. Gazzinelli RT, Mendonca-Neto R, Lilue J, Howard J, Sher A: **Innate**
• **resistance against *Toxoplasma gondii*: an evolutionary tale of mice, cats, and men**. *Cell Host Microbe* 2014, **15**:132-138.
The first attempt to attribute the presence of distinctive TLR and IRG proteins in mice to the status of mice as evolutionarily significant hosts.

40. Tosh KW, Mittereder L, Bonne-Annee S, Hieny S, Nutman TB, Singer SM, Sher A, Jankovic D: **The IL-12 response of primary human dendritic cells and monocytes to *Toxoplasma gondii* is stimulated by phagocytosis of live parasites rather than host cell invasion**. *J Immunol* 2016, **196**:345-356.

41. Morger J, Bajnok J, Boyce K, Craig PS, Rogan MT, Lun ZR, Hide G,
• Tschirren B: **Naturally occurring Toll-like receptor 11 (TLR11) and Toll-like receptor 12 (TLR12) polymorphisms are not associated with *Toxoplasma gondii* infection in wild wood mice**. *Infect Genet Evol* 2014, **26**:180-184.
This paper reveals no sign of a correlation between sequence polymorphisms in TLR11/12 and probability of infection by *T. gondii* in wild wood mice. This is the first paper to attempt an analysis of the evolutionary significance hypothesis in an ecological context.

42. Lilue J, Muller UB, Steinfeldt T, Howard JC: **Reciprocal virulence and resistance polymorphism in the relationship between *Toxoplasma gondii* and the house mouse**. *Elife* 2013, **2**:e01298.

43. Hunter CA, Sibley LD: **Modulation of innate immunity by *Toxoplasma gondii* virulence effectors**. *Nat Rev Microbiol* 2012, **10**:766-778.

44. Reese ML, Shah N, Boothroyd JC: **The Toxoplasma pseudokinase ROP5 is an allosteric inhibitor of the immunity-related GTPases**. *J Biol Chem* 2014, **289**:27849-27858.

45. Behnke MS, Khan A, Lauron EJ, Jimah JR, Wang Q, Tolia NH,
•• Sibley LD: **Rhoptry proteins ROP5 and ROP18 are major murine virulence factors in genetically divergent south american strains of *Toxoplasma gondii***. *PLoS Genet* 2015, **11** e1005434.
An exceptional study proving beyond doubt that the virulence of many *T. gondii* strains from S. America, is due to the activity of novel alleles of ROP5 and ROP18. It follows with high probability from this study that IRG proteins are targeted for inactivation also by these strains. It will be interesting to discover for which intermediate host species these highly active virulence factors are adaptive.

46. Bernstein-Hanley I, Coers J, Balsara ZR, Taylor GA, Starnbach MN, Dietrich WF: **The p47 GTPases Igtp and Irgb10 map to the *Chlamydia trachomatis* susceptibility locus Ctrq-3 and mediate cellular resistance in mice**. *Proc Natl Acad Sci U S A* 2006, **103**:14092-14097.

47. Miyairi I, Tatireddigari VR, Mahdi OS, Rose LA, Belland RJ, Lu L, Williams RW, Byrne GI: **The p47 GTPases Iigp2 and Irgb10 regulate innate immunity and inflammation to murine *Chlamydia psittaci* infection**. *J Immunol* 2007, **179**:1814-1824.

48. Ferreira-da-Silva Mda F, Springer-Frauenhoff HM, Bohne W, Howard JC: **Identification of the microsporidian *Encephalitozoon cuniculi* as a new target of the IFNgamma-inducible IRG resistance system**. *PLoS Pathog* 2014, **10**:e1004449.

49. Demar M, Ajzenberg D, Maubon D, Djossou F, Panchoe D, Punwasi W, Valery N, Peneau C, Daigre JL, Aznar C, Cottrelle B, Terzan L, Dardé ML, Carme B: **Fatal outbreak of human toxoplasmosis along the Maroni River: epidemiological, clinical, and parasitological aspects**. *Clin Infect Dis* 2007, **45**:e88-95.

50. Yang H, Wang JR, Didion JP, Buus RJ, Bell TA, Welsh CE, Bonhomme F, Yu AH, Nachman MW, Pialek J *et al.*: **Subspecific origin and haplotype diversity in the laboratory mouse**. *Nat Genet* 2011, **43**:648-655.

51. Blanchard N, Gonzalez F, Schaeffer M, Joncker NT, Cheng T, Shastri AJ, Robey EA, Shastri N: **Immunodominant, protective response to the parasite *Toxoplasma gondii* requires antigen processing in the endoplasmic reticulum**. *Nat Immunol* 2008, **9**:937-944.

52. Frickel EM, Sahoo N, Hopp J, Gubbels MJ, Craver MP, Knoll LJ, Ploegh HL, Grotenbreg GM: **Parasite stage-specific recognition of endogenous *Toxoplasma gondii*-derived CD8+ T cell epitopes**. *J Infect Dis* 2008, **198**:1625-1633.