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PERSPECTIVE

Galleria mellonella as a model host for microbiological and toxin research

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ABSTRACT

Mammals are widely used by microbiologists as a model host species to study infectious diseases of humans and domesticated livestock. These studies have been pivotal for our understanding of mechanisms of virulence and have allowed the development of diagnostics, pre-treatments and therapies for disease. However, over the past decade we have seen efforts to identify organisms which can be used as alternatives to mammals for these studies. The drivers for this are complex and multifactorial and include cost, ethical and scientific considerations. *Galleria mellonella* have been used as an alternative infection model since the 1980s and its utility for the study of bacterial disease and antimicrobial discovery was recently comprehensively reviewed. The wider applications of *G. mellonella* as a model host, including its susceptibility to 29 species of fungi, 7 viruses, 1 species of parasite and 16 biological toxins, are described in this perspective. In addition, the latest developments in the standardisation of *G. mellonella* larvae for research purposes has been reviewed.

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Introduction

For the past two decades, microbiologists have sought alternatives to mammals for studying the molecular basis of virulence and for testing antimicrobial drugs. In April's issue of *Virulence*, Tsai et al.[1] reviewed the extensive body of literature which reports the value of *Galleria mellonella* (Greater wax moth) larvae as a model for investigating bacterial pathogens. The authors highlight many of the attractive features of this model: when compared with mammals, *G. mellonella* larvae are cheaper and easier to maintain, they do not require specialized laboratories or equipment and work with *G. mellonella* does not require ethical approval. Unlike many alternative models *G. mellonella* can be maintained at 37°C. We also think an important feature of this model is the ease with which the larvae can be injected with precise doses of pathogen, allowing the relative virulence of strains and mutants to be compared. As Tsai et al[1] point out, these features of the *G. mellonella* model should even allow high throughput screens to be carried out on a scale that would not be ethically or financially possible using mammals. In this perspective we highlight some of the applications of the *G. mellonella* model beyond work with bacterial pathogens, including fungal, viral, microbiota and toxin research. We also comment on some of the key points raised by Tsai et al[1] and which they highlight as barriers to the wider use of this model by the community including the requirement for standardised Galleria and the lack of a genome sequence.

G. mellonella *as a model to study fungal pathogens*

G. mellonella was first described as a model for studying human fungal pathogens in the yeast *Candida albicans*[2] where larval susceptibility to fungal challenge was used to distinguish between pathogenic and non-pathogenic *C. albicans* strains.[2] *G. mellonella* has since been used as a model to distinguish between the virulence of different strains of fungi[2,3] and their relative virulence at 30°C and 37°C.[4]

G. mellonella has also been useful to identify virulence determinants by screening for attenuation of mutants. The results of these studies correlate well with studies performed in mice as well as data from infected humans.[5] For example, a positive correlation between the virulence of *C. albicans* mutants when tested in Balb/c mice or in *G. mellonella* larvae has been observed.[6] In the human fungal pathogen *Aspergillus fumigatus* deletion mutants of *cpcA, sidA, sidF* and *paba* were avirulent in *G. mellonella* while deletion mutants of *sidC* and *sidD* demonstrated attenuated virulence. These results were comparable with data derived from assessments made in mammalian models such as mice.[5] These studies show that pre-screening of *C. albicans and A. fumigatus*

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virulence mutants in *G. mellonella* may significantly reduce the number of mammalian animals needed to assess changes in virulence.

G. mellonella has subsequently been used to study other fungal pathogens including *Aspergillus fumigatus*,[5,7] *Histoplasma capsulatum*,[8] *Paracoccidioides lutzii, Fusarium species*[9,10] and other *Cryptococcus* species[11] (Full list of fungal species tested in *G. mellonella* is summarised in Table 1).

As well as studying virulence in *C. albicans*[12-15] the larvae have been used as a model to study tissue invasion capabilities between biofilm producing and non-producing isolates,[3] the role of the filamentation phenotype in virulence[16] and as a model to screen for efficacy of antifungal compounds.[4,17]

Table 1. Fungal species tested in *G. mellonella*.

Fungi	Assay	Reference
Aspergillus flavus	Virulence	39
Aspergillus fumigatus	Virulence	5,7
Aspergillus terreus	Virulence, antibiotic resistant and sensitive isolates	40
Candida Africana	Virulence, assessing antifungal compounds	41
Candida albicans	Biofilm formation, invasion, filamentation assay, virulence	2,3,12, 16,42,43
Candida glabrata	Virulence	43
Candida krusei	Virulence, assessing antifungals compounds	43,44
Candida metapsilosis	Virulence	45
Candida orthopsilosis	Virulence	45
Candida parapsilosis,	Virulence	43,45
Candida tropicalis	Virulence, assessing antifungals compounds	43,46
Conidiobolus coronatus (non-human pathogen)	Insect immunology, assessing fungal compunds	47,48
Cryptococcus gattii	Virulence	11
Cryptococcus neoformans	Virulence, host immune responses, assessing antifungal compounds	4, 49,50
Fusarium cerealis	Virulence, assessing antifungal compounds	9
Fusarium culmorum (non-human pathogen)	Virulence	7
Fusarium oxysporum	Virulence	7,10
Fusarium proliferatum (non-human pathogen)	Virulence	7
Fusarium solani	Virulence	7
Fusarium verticillioides	Virulence	7
Histoplasma capsulatum	Virulence	8
Madurella mycetomatis (non-human pathogen)	Induce grain formation	51
Metarhizium robertsii (non-human pathogen)	Virulence	52
Paracoccidioides brasiliensis	Virulence	53
Paracoccidioides lutzii	Virulence	8
Penicillium marneffei	Virulence, phagocytosis	54
Pneumocystis murina	Virulence	55
Rhizopus coincides	Virulence, thermotolerance	56
Saccharomyces cerevisiae	Virulence	2
Scedosporium aurantiacum	Virulence, substrate utilization	57
Trichosporon asteroids	Virulence, Assessing antifungal compounds	58
Trichosporon inkin	Virulence, Assessing antifungal compounds	58
Trichosporon asahii	Virulence, Assessing antifungal compounds	58

G. mellonella *to study virus*

As well as a model for studying bacterial and fungal pathogens, there are a few reports of the use of *G. mellonella* to investigate viral disease, and not surprisingly most of these studies have involved insect pathogenic viruses such as Tipula iridescent virus (TIV)[18] and Invertebrate Iridescent Virus 6 (IIV6).[19] In some cases the larvae have been challenged with virus, in others haemocytes isolated from the larvae have been infected (A full list of viruses tested in *G. mellonella* summarised in Table 2). The *Galleria* model has not, so far, been shown to be suitable for research into viral pathogens of mammals. This may be because insect cells are incubated at 25 – 30°C which may not support the growth of mammalian viruses. In addition, viruses often show tropism toward cells bearing specific receptors that may not be shared by mammalian and insect cell lines.

G. mellonella *to study toxins*

In a limited number of studies preparations from either bacteria or fungi have been injected into *G. mellonella* to study their toxicity. In many cases the toxins studied are known to be insecticidal and *G. mellonella* larvae provide a good model to further investigate toxicity. For example, the bacteria *Pseudomonas fluorescens* is able to protect crop plants from fungal root disease. However, insecticidal toxin (Fit toxin) produced from some strains of *P. fluorescens (CHA0 and Pf-5)* has been shown to be a potent insect toxin. A study by Pechy-Taar and colleagues showed that low doses of *P. fluorescens* were able to kill the larvae while a deletion mutant of the Fit toxin was significantly attenuated.[20] To understand the modes of action of toxins produced by pathogenic fungi of insects such as cyclosporins, beauverolides and destruxins[21,22] the use of *G. mellonella* has moved beyond a whole animal system to include the preparation of cell lines to study the effect of fungal toxins on the performance of immune competent hemocytes *in vitro*. Quantification of the effect of these toxins on attachment, spreading and phagocytic activity has been measured.

Table 2. Virus' tested in *G. mellonella*.

Virus	Assay	Reference
Bovine herpes simplex virus-1 (BHSV-1)	Nodulation	59
Densonucleosis virus	Infectivity	60
Invertebrate iridescent virus 6	Infectivity	19
Iridovirus	Infectivity	60
Mycovirus	Infectivity	61
Nodamura virus	Infectivity, muscle cell modification	62
Tipula iridescent virus TIV	Infectivity, gamma radiation responses	18,63

With the development of *G. mellonella* as a model to investigate the roles of toxins in human disease similar methods have started be applied to explore the modes of action of toxins from pathogenic fungi and bacteria of humans. For example, the fumagillin toxin from *Aspergillus fumigatus* has been shown to suppress the cellular immune response of the *G. mellonella* larvae by inhibiting the action of haemocytes and this made the larvae more susceptible to a subsequent challenge with *A. fumigatus*.[23] In another study extracellular gelatinase (GelE) and serine protease (SprE) produced by *Enterococcus faecalis* were injected into *G. mellonella*.[24] GelE degraded antimicrobial peptides such as cecropin produced by the larvae and this finding stimulated subsequent studies showing that GelE was able to hydrolyse the C3a component of complement and mediate the degradation of the α chain of C3b. In addition, the protease SprE produced by *E. faecalis* showed no virulence in either insect haemolymph or in human serum. However, larvae are resistant to toxins such as the *Clostridium perfringens* α- and epsilon-toxins (unpublished data) which are active against mammalian cells. Considering that *C. perfringens* epsilon-toxin binds to specific cellular receptors this finding is not surprising. However, as a membrane active phospholipase C the α-toxin is active against many cells types. These studies show that *G. mellonella* can be used to study some, but not all, extracellular compounds, such as toxins, of both bacteria and fungi.

Applications of G. mellonella *to study microbiota*

G. mellonella has been used as a model host to understand the composition of the microbiota of holometabolous insects during metamorphosis.[25] However, normal microbiota has been implicated as a critical defense against invading pathogens in humans and there is a growing body of evidence supporting a role for *G. mellonella* as a model host in which to study these interactions.[26] Not only do insect and mammalian gastrointestinal tracts share similar tissues, anatomy, and physiological functions but the microvilli of the Lepidopteran midgut contain Enterococci, Lactobacilli, and Clostridial Firmicutes that are also found in the intestinal microvilli of mammals.[27-29] The gut microbiota of insects are solely maintained by the innate immune system and it has been suggested that microbial diversity of the microbiota may be responsible for specific immune phenotypes.[30] Co evolution of the innate immune response and microbiota can therefore be investigated in insect models without cross-talk with the adaptive immune responses of mammals and *G. mellonella* has been established as a model in which to study co evolution.[31]

Standardisation of G. mellonella *larvae*

Tsai et al have identified the lack of standardised larvae as a significant barrier to the wider adoption of this model. G. *mellonella* have been commercially available as food for captive reptiles and birds and as fishing bait, and larvae bred for these purposes have been widely used in research. Preliminary studies with standardised *G. mellonella* larvae (TruLarv™[32]) suggest that they provide statistically more reproducible results. Therefore, further studies with these standardised larvae are now warranted. In addition, a program to genome sequence these standardised larvae is ongoing and the data will be released into the public domain when completed.

In mammalian model hosts, a variety of end points which are guided by welfare considerations are employed to assess response to a pathogen or compound. End points in *G. mellonella* infection models include survival rate, which can be assessed up to 5 d post infection, or longer with some fungal pathogens, facilitating the calculation of a maximum half lethal dose (LD50); expression of antimicrobial proteins in response to infection; production of lactate dehydrogenase as a marker of cell damage and biophotonic imaging to measure proliferation of bioluminescent microorganisms responsible for larval infection.[33-37] A pathological scoring system was recently proposed by Loh et al. [38] in which an assessment of larval mobility, cocoon formation, melanisation and survival was used to assess larval health.

In conclusion, the relevance of *G. mellonella* as a model host for bacterial pathogens and for screening antimicrobial compounds has now been firmly established. Now that *G. mellonella* has been successfully established as a model host for microbiology, new applications are being tested. For example, *G. mellonella* as an eco toxicological test organism to study the effects of natural or man-made chemicals, as a model host for microbiota research and as a model host for studying toxins. Limitations associated with *G. mellonella* are currently being addressed, both through the development of standardised larvae for research, genome sequencing projects and the development of pathology scores for more sophisticated end points. The power of *G. mellonella* as a model host lies not only in its ability to improve the efficiency of research through decreased cost and time associated with the use of mammalian model hosts, but also in the ability to increase the scale and therefore the statistical power of experiments. Also, as results have been shown to correlate well with those in mammals, *G. mellonella* provides a powerful and adaptable initial screen to reduce reliance on experimental mammals.

Disclosure of potential conflicts of interest

The authors Olivia L. Champion and Richard W. Titball have an interest in BioSystems Technology Limited.

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