Paving the way to acceptance of *Galleria mellonella* as a new model insect

Andrew P. Desbois* and Stuart McMillan

Marine Biotechnology Research Group, Institute of Aquaculture, School of Natural Sciences,

University of Stirling, United Kingdom

*Author for correspondence (ad54@stir.ac.uk)

The larva of the greater wax moth *Galleria mellonella* is an alternative host used commonly in studies of microbial infection and innate immunity. Indeed, this insect host is often used when quantifying or comparing the virulence of bacterial and fungal pathogens of vertebrates and it has been used successfully to establish the importance of microbial virulence factors and to determine the relative virulence of different isolates of the same species. [1-5] The recent popularity of *G. mellonella* as an alternative host system stems from numerous benefits, including the ability to perform experiments at a range of temperatures including human body core temperature; the technical simplicity of establishing infections by various routes such as through feeding, topical application or injection; the convenient size of the insect, which means it is large enough to permit simple injection of inoculums or chemicals but small enough to require little space in the laboratory; the ability to assess the efficacy and toxicity of antimicrobial therapies; and the ease and reliability with which these insects can be sourced in their final instar stage from commercial suppliers. [6] It has also found approval amongst many researchers due to the favourable reproducibility between experiments in the same laboratory. Nevertheless, relatively small variations in susceptibility to infection can occur between batches of larvae from the same supplier and such variation probably arises from factors such as age, size and nutritional status on receipt; conditions encountered during transit to the laboratory; and the presence of any underlying natural infections. These issues are largely uncontrollable when purchasing larvae from a commercial supplier but on reaching the laboratory standardised pre-experimentation storage conditions can improve reproducibility between studies. In recent years the Kavanagh group have raised awareness for the role of a number of variables during storage that require consideration to ensure optimal reproducibility when experimenting with this insect, and factors influencing *G. mellonella* susceptibility to infections include physical stress,[7] incubation temperature[8] and access to food.[9] In this edition of *Virulence*, the Kavanagh group report that larvae become increasingly susceptible to infection by pathogens as laboratory storage time increases, highlighting the need to consider this parameter when using the *G. mellonella* model. Browne et al.[10] elaborate further in the study and relate this observation to a

reduction in the total abundance of haemocytes that function in immune defence against pathogens and changes in the relative flux of metabolic pathways. Interestingly, the number of haemocytes after 3 weeks of incubation was approximately half that compared to the population at one week, while qualitative changes in the relative abundance of the various types of haemocytes were also reported.[10] Both these factors probably contribute to reduced immune capacity and thus increased susceptibility to infection.

The Browne et al. study[10] and other related works raise awareness of ways to reduce inter-experimental variability and may help to standardise methods and permit more meaningful comparisons between certain types of experiment. Moreover, studies of this nature are underpinning the development of this alternative host and, importantly, serving as part of a broader worthwhile pursuit to secure greater recognition for the value of this insect to infection biology.[11] There is a desire amongst the growing community of researchers using this insect for it to achieve a status similar to other accepted model organisms for infection studies like *Drosophila melanogaster* or *Caenorhabditis elegans*, and this journal in particular is working hard to raise the profile of this and other alternative hosts. Certainly there is justification for another model insect to complement the existing models and approaches, and an opportunity exists for *G. mellonella* to be developed to fill this niche. *G. mellonella* studies would permit deeper insight into immune functioning because we know already that fundamental differences exist compared to the immune responses of *D. melanogaster* and deeper understanding could be gleaned from studying both organisms.[12] In addition to the favourable attributes mentioned above, *G. mellonella* is already associated with characteristics seen as desirable for a model organism including a long and rich history in the academic literature through studies on physiology, reproduction biology and innate immune responses. Thus, *G. mellonella* seems a reasonable choice to pursue as a model species especially given the rapid rise in popularity for using this insect in infection studies.[6]

Looking to the future, if *G.mellonella* is to be established as an accepted model species, particularly for infection studies, certain key advancements need to be made (Table). Top priority has to be the completion and publication of a fully annotated genome sequence, as well as integrated database resources for access to genomic, transcriptomic and proteomic information[13] akin to the situation for other model organisms with Flybase[14] and Wormbase.[15] Following on from this, it would be desirable to determine the genetic diversity of the *G.mellonella* strains currently being used in experiments, and this should result in the defining of standard strains and perhaps the creation of a commercial strain resource. Of course, access to well-defined insect strains from trusted sources reared under standard conditions can be expected to reduce experimental variability even further. Beyond these requirements, the wish list of the *G. mellonella* research community includes the development of an improved molecular biology toolkit to enable the reliable genetic manipulation of this insect. Ultimately, the creation of a collection of knockout strains would be an important addition to the scientific community and this would be a valuable resource for many researchers even those working in fields beyond infection biology. Meeting the requirements of this wish list will further increase the popularity of this model, which should help to unlock the tremendous potential of this alternative host for providing deeper insight into the innate immune response including the key networks and pathways; the mechanisms employed by pathogens to avoid and surmount the innate immune response; and the interplay between the host and pathogen during infection. The number of *G. mellonella* users is growing rapidly and there is momentum towards achieving greater recognition for this alternative host, and prompt collective action should help us to overcome the barriers currently preventing wider acceptance of this insect as a model organism.

About the authors

APD is Lecturer in Marine Biotechnology at the Institute of Aquaculture, University of Stirling, UK.

SM is a graduate student supported by a University of Stirling Collaborative Research Studentship in partnership with the Centre for Environment, Fisheries and Aquaculture Science (Weymouth, UK).

Table. Important resources currently unavailable to *Galleria mellonella* user community.

Resource
Access to fully annotated genome(s)
Integrated database for *Galleria mellonella* information
Availability of well-defined strains, including knockouts
Development of a comprehensive molecular biology toolkit

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