PHILOSOPHICAL TRANSACTIONS B

royalsocietypublishing.org/journal/rstb

Review

Cite this article: Seppälä O, Çetin C, Cereghetti T, Feulner PGD, Adema CM. 2021 Examining adaptive evolution of immune activity: opportunities provided by gastropods in the age of 'omics'. *Phil. Trans. R. Soc. B* **376**: 20200158.
https://doi.org/10.1098/rstb.2020.0158

Accepted: 23 November 2020

One contribution of 15 to a Theo Murphy meeting issue 'Molluscan genomics: broad insights and future directions for a neglected phylum'.

Subject Areas:
evolution, genomics, immunology

Keywords:
gastropoda, heritability, immune function, immunocompetence, *Lymnaea stagnalis*

Author for correspondence:
Otto Seppälä
e-mail: otto.seppaelae@uibk.ac.at

THE ROYAL SOCIETY PUBLISHING

Examining adaptive evolution of immune activity: opportunities provided by gastropods in the age of 'omics'

Otto Seppälä[1], Cansu Çetin[2,3], Teo Cereghetti[2,3], Philine G. D. Feulner[4,5] and Coen M. Adema[6]

[1]Research Department for Limnology, University of Innsbruck, Mondsee, Austria
[2]Department of Aquatic Ecology, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland
[3]Institute of Integrative Biology, ETH Zürich, Zürich, Switzerland
[4]Department of Fish Ecology and Evolution, Centre of Ecology, Evolution and Biogeochemistry, Swiss Federal Institute of Aquatic Science and Technology, Kastanienbaum, Switzerland
[5]Division of Aquatic Ecology and Evolution, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland
[6]Department of Biology, Center for Evolutionary and Theoretical Immunology, University of New Mexico, Albuquerque, NM, USA

OS, 0000-0001-7902-3069; CMA, 0000-0002-6250-5499

Parasites threaten all free-living organisms, including molluscs. Understanding the evolution of immune defence traits in natural host populations is crucial for predicting their long-term performance under continuous infection risk. Adaptive trait evolution requires that traits are subject to selection (i.e. contribute to organismal fitness) and that they are heritable. Despite broad interest in the evolutionary ecology of immune activity in animals, the understanding of selection on and evolutionary potential of immune defence traits is far from comprehensive. For instance, empirical observations are only rarely in line with theoretical predictions of immune activity being subject to stabilizing selection. This discrepancy may be because ecoimmunological studies can typically cover only a fraction of the complexity of an animal immune system. Similarly, molecular immunology/immunogenetics studies provide a mechanistic understanding of immunity, but neglect variation that arises from natural genetic differences among individuals and from environmental conditions. Here, we review the current literature on natural selection on and evolutionary potential of immune traits in animals, signal how merging ecological immunology and genomics will strengthen evolutionary ecological research on immunity, and indicate research opportunities for molluscan gastropods for which well-established ecological understanding and/or 'immune-omics' resources are already available.

This article is part of the Theo Murphy meeting issue 'Molluscan genomics: broad insights and future directions for a neglected phylum'.

1. Introduction

Parasites (here referring to both micro- (e.g. viruses and bacteria) and macroparasites (e.g. helminths)) present a severe threat to free-living organisms, including molluscs, by reducing their survival and fecundity. Such adverse fitness effects can, for example, influence the evolution of host life-histories [1,2] and drive sexual selection [3,4]. Furthermore, if host individuals fail to resist infections and/or eliminate them after establishment, parasite prevalence in a host population may rapidly increase, eventually crashing it (reviewed in [5]). Owing to complicated species interactions in natural communities, reduced host population density may have broad ecological consequences, for instance, by altering resource–consumer interactions, and also jeopardize vital ecosystem

services (e.g. [6,7]). Moreover, although biomedical science has been able to eliminate several disease-causing agents (mostly viruses and bacteria), parasites are still one of the most common causes of death in humans and sources of economic loss in agriculture (e.g. [8,9]). The threat of disease is even expected to increase in the future because of the continuous emergence of new disease-causing agents [10,11], the evolution of drug resistance (reviewed in [12,13]) and biological invasions (reviewed in [14]). Therefore, to create projections of the risks that parasites impose, a crucial element to understand is if and how host populations may evolutionarily adapt to parasitism.

Several factors are known to play essential roles in determining host susceptibility to infections, including host and parasite genetics (e.g. [15–17]), host gender (e.g. [2,18]), host age (e.g. [19,20]), host nutritional state (e.g. [21,22]), host behaviour [23,24] and environmental conditions (e.g. [25,26]). Many of these effects arise from differences in host immune function, which is the primary physiological barrier against infections (reviewed in [27]). Therefore, understanding the outcomes of host–parasite interactions, and thus disease outbreaks in nature, requires detailed knowledge on the evolutionary responses of immune defence traits to parasite-mediated selection. The host immune function has recently become an important research topic in several fields of ecology and evolutionary biology (see [28]). This development has given rise to the interdisciplinary field of ecological immunology (or ecoimmunology; see [29]) that has proven to be highly useful when investigating the evolution of host immune defence traits in natural systems (reviewed in [30]). That research can be expected to be of great help when evaluating the role of evolution in determining future disease outbreaks.

Ecological immunologists typically focus on quantitative immune defence traits such as the amount of end products of immune cascades that are controlled by several genes. This approach is chosen because many immunological processes, especially in invertebrates, consist of traits that are not strictly specific to certain parasites [31] and are likely to evolve through selection on additive genetic variance (e.g. [32–34]) rather than frequency-dependent selection (reviewed in [35]). Adaptive evolution of quantitative traits requires that phenotypic trait variation reflects fitness variation (i.e. traits are subject to natural selection) and that it is at least partly heritable (i.e. traits show additive genetic variation; [36]). In this article, we briefly review earlier empirical work on both natural selection on and genetic variation in immune defence traits across animal systems to present the general state of research in the field. Then, we discuss how we believe the recent development in the fields of genomics and transcriptomics could support future investigations in the evolutionary ecology of host immune activity. Lastly, we review the state of research focusing on the evolution of immune activity in molluscs and propose how the rapidly expanding genomics and transcriptomics resources in this group of organisms (e.g. [37–39]) could be of great help strengthening future ecoimmunological research.

2. Natural selection on immune activity

The first requirement for the adaptive evolution of a phenotypic trait is that it is subject to natural selection. From the potential forms of selection on quantitative traits [36], positive directional (i.e. the highest trait values lead to the highest fitness) and stabilizing selection (i.e. intermediate trait values lead to the highest fitness) are considered most relevant for immune traits. First, since the function of the immune system is to prevent and eliminate infections by harmful (i.e. virulent) parasites, a strong immune system can be assumed to increase fitness and evolve as a response to parasitism (e.g. [40,41]). However, the immune defence is typically energetically costly to maintain and use (reviewed in [42,43]), which can lead to trade-offs between immune function and life-history traits (e.g. [44,45]), as well as between different immunological mechanisms [32]. Therefore, strong immune defence (and subsequent low parasite abundance) does not necessarily lead to the highest fitness. In fact, theoretical models predict host immune function to evolve under stabilizing selection when immune activity is costly to maintain and use (reviewed in [46]). Contrary to the theoretical predictions, empirical studies that are mainly conducted using birds (a few studies exist on mammals, reptiles and insects) typically suggest positive directional selection on immune function through its positive effects on survival and fecundity (reviewed in [46]). A few studies report stabilizing or even negative directional selection on immune defence traits [47–50]. Owing to the predicted costs associated with immune function (see above), evidence for positive directional selection is surprising and may arise from challenges to identify and measure appropriate parameters of host immune function as well as fitness components.

The above studies on natural selection on immune function typically focus on measuring the end products of one or a few immunological cascades (but see [51]). However, the immune system is formed from several different components that are effective against different types of parasites (reviewed in [27,52]). For example, the immune system of the fruit fly shows specific responses towards Gram-positive bacteria, Gram-negative bacteria and fungi (e.g. [53,54]), and similar specificity has been seen in other taxa (e.g. [38,55]). Additionally, immunological pathways consist of several steps (recognition, signalling, effectors) that are crucial for successful immune responses, and different components and steps of the immune response may be traded-off with different physiological, life-history and/or immune defence traits [32,38,56,57]. Furthermore, the activity of different immunological mechanisms, their relative contribution to a successful defence and the costs related to high immune activity may vary over space and time. This variation could depend on, for example, infection risk in the environment, the type of parasites the hosts are exposed to and environmental conditions that determine the expression of trade-offs [46]. These factors make predicting evolutionary forces that shape immune function in natural populations very difficult when only a narrow subset of immune traits is examined to quantify selection. Therefore, although ecoimmunological studies can give detailed estimates about the evolution of specific immune traits, they are not as successful at providing a general understanding of the evolution of immune activity at the level of the whole immune system.

The recent development in transcriptomics (see [58,59]) provides excellent opportunities to overcome the above-mentioned challenges when investigating the evolution of organisms' immune activity. In general, trait evolution may depend more strongly on variability in gene expression than on variation in protein-coding sequences [60,61]. In fact, the

genetic basis of transcription and its evolution under natural selection is well demonstrated in yeast (e.g. [62,63]), fruit fly (e.g. [64,65]) and fish (e.g. [66,67]). For instance, a study on killifish *Fundulus heteroclitus* identified 13 genes with variation in transcription among natural populations that indicate thermal adaptation across a latitudinal gradient [66]. Such studies show that gene expression can be a meaningful predictor of individuals' performance and could be used in the quantitative genetic (i.e. statistical genetic) framework as a 'phenotype' (reviewed in [68]).

Transcriptomics has become especially fruitful in evolutionary ecology in the era of the rapid development of high-throughput gene expression analysis technologies. Currently, it is possible to measure the transcription of numerous genes selected across the whole genome in a very cost- and time-efficient manner (e.g. [69]). In ecological immunology, this allows using transcription of a broad range of genes that cover different immunological pathways and steps of immunological cascades (i.e. recognition, signalling, effectors) to comprehensively quantify the 'immune phenotypes' (*sensu* [70]) of individuals. However, ecoimmunological research is still rarely conducted at the gene expression level. So far, condition dependence of immune activity [71], genetic specificity between hosts and parasites [72] and immune priming [73,74] have been investigated by quantifying transcription in bumblebee and red flour beetle. Those studies have hugely benefitted from the detailed examination of different components of the host immune system provided by transcriptomics technologies. To our knowledge, however, gene expression analysis has not been incorporated in earlier studies on natural selection on immune function.

3. Evolutionary potential of immune activity

The second requirement for adaptive trait evolution is that the traits under selection can respond to it. Specifically, fitness-related traits need to show heritable genetic variation [36]. Therefore, understanding the genetic architecture of and the extent and type of genetic variation in phenotypic traits is indispensable for understanding their evolution [75]. In fact, if and how natural populations can evolutionarily respond to natural selection is one of the main topics in current evolutionary ecological research. Estimating quantitative genetic parameters such as additive genetic variance and covariance of traits is an efficient approach for testing whether or not natural populations can evolve through adaptation, and how fast this process can be (reviewed in [76,77]). This is especially important because in many systems, natural populations do not respond to the observed selection, or their responses differ from the predictions based on selection (e.g. [78,79]). The above approach is highly relevant also in the case of immune defence traits. However, despite wide interest on the evolutionary potential of immune traits (e.g. [15,32,34,80]) this information is mostly lacking from natural populations (but see [81–84]). The scarcity of such knowledge prevents predicting the evolutionary responses of host defences to parasitism.

One main reason for the poor understanding of the evolutionary potential of defence against parasites is that earlier genetic research on immune function has been largely divided into two separate fields: molecular immunogenetics and quantitative genetics. Molecular immunogenetics focuses on describing genetic mechanisms underlying the structure and functioning of individual components of the immune system from a medical perspective. Such information has, of course, important implications in society, but they rarely shed light on ecological and evolutionary relevance of immune function. The latter is because those studies are typically conducted using specific strains of model organisms for biomedical research and do not consider natural genetic variation (e.g. specific mouse strains [85,86]). Quantitative genetic studies, on the other hand, examine genetic variation by focusing on natural populations or at least laboratory stocks that originate from the field. However, many quantitative genetic studies also are limited to laboratory conditions owing to the need for controlled breeding designs that estimate quantitative genetic parameters such as heritability (i.e. the proportion of trait variation arising from breeding values) and genetic correlation. Such studies are especially common in invertebrates (e.g. [32–34]).

The main limitation of breeding designs conducted under laboratory conditions is that the estimated quantitative genetic parameters may not reflect their actual values under natural conditions. This discrepancy is likely because, for example, trait heritability and genetic correlations often depend on the environmental conditions under which they are estimated (reviewed in [87,88]). Dependence on environmental conditions is because several environmental factors such as resource availability and ambient temperature can affect variation in trait values among individuals, as well as the expression of trade-offs. Therefore, quantitative genetic studies are most useful in study systems in which social pedigrees over many generations are available from natural populations (mainly mammals and birds; reviewed in [89]). To our knowledge, such studies on immune defence have only been conducted in Soay sheep [84] and a few bird species (e.g. [81–83]). The rarity of such studies is likely to be because collecting pedigree data in natural populations is always demanding and practically impossible in many study systems (e.g. invertebrates). Furthermore, similarly to the studies on natural selection on immune activity described above, quantitative genetic studies on immune function focus on a few phenotypic immune traits that reflect the amount of end products of immune cascades (e.g. [32–34]). Thus, quantitative genetic studies are often not successful at predicting the evolution of the immune system as a whole and would greatly benefit from the integration of transcriptomics to expand the collection of measured immune traits at the gene expression level. To our knowledge, such an analysis on the genetic architecture (i.e. variance components) of the expression of several immune traits has not yet been conducted.

In the field of quantitative genetics, interest in using genomics tools when examining the heritability of phenotypic traits is currently increasing. Using genomics methods allows, for instance, genotyping of individuals with high marker density across the whole genome (e.g. single nucleotide polymorphism (SNP) genotyping using SNP chips or restriction site-associated DNA sequencing (RAD-seq) [90,91]) to estimate relatedness among individuals in natural populations. The advantage of these methods is that they measure the realized genomic relatedness based on the proportion of genome identity-by-state between all pairs of individuals. Such estimates can differ significantly from the expected values of identity-by-descent provided by pedigrees [92]. These methods have been used to improve the available

pedigree information, for example, in the great tit [93,94] and Soay sheep [95,96] populations when calculating quantitative genetic parameters for morphological and life-history traits. The obtained genetic data have proven to be highly useful by improving parameter estimates when compared with those that use only pedigree information [95,97,98]. Additionally, RAD-seq data have been used to estimate the heritability of body mass in roe deer without any pedigree information [99]. However, only one study on Soay sheep [84] has focused on immune traits by using a high-density SNP chip to build a genomic relatedness matrix for quantitative genetic analyses. It is, however, important to note that heritability estimated via SNP data is expected to be lower than narrow-sense heritability calculated, for example, from pedigree data. This difference is because of the imperfect tagging of the causal variants by SNPs. Because SNP genotyping typically focuses on common alleles (greater than 1% frequency), SNP heritability does not capture the contribution of rare SNPs to trait variation [100].

The above genotyping approaches provide additional opportunities for more detailed investigation of the genetic architecture of the examined traits. For instance, marker-based partitioning of phenotypic trait variation across chromosomes helps to estimate whether the traits of interest are polygenic or not [93,94,96,101]. If the contribution of different chromosomes on trait heritability depends on their size, the trait should be polygenic. However, if only one chromosome (not necessarily the largest) explains most of the trait heritability, then the trait is likely to be determined by a small number of genes with large effects. Furthermore, identifying candidate loci underlying phenotypic trait variation (e.g. using genome-wide association studies (GWAS) [102]) allows examining covariation in their phenotypic effects [103]. Because of these advantages, the interest in using methods like GWAS in natural populations of wild species is increasing in the field of quantitative genetics (e.g. [96,104,105]). In our opinion, however, the greatest benefit of 'molecular quantitative genetics' is that it enables studies on natural populations of invertebrates and plants that are currently severely underrepresented in this field owing to the lack of social pedigree information [89].

4. Natural selection on and evolutionary potential of immune activity in molluscs

In molluscs, natural selection on immune activity has been examined in the great pond snail, *Lymnaea stagnalis*. In a field study by Langeloh *et al*. [106], snails from a genetically diverse laboratory stock were maintained in enclosures in a lake for several weeks. The stock population experimental snails originated from was initiated by interbreeding individuals from several natural populations to increase genetic and phenotypic variation among individuals because snail populations in the field often show low genetic diversity [107]. This way, the risk of limited phenotypic variation preventing the detection of stabilizing selection aimed to be minimized (see [46]). Over the course of the study, snails' immune activity (antibacterial activity and phenoloxidase (PO)-like activity of haemolymph), as well as fitness components such as survival and fecundity, were followed. The results indicated positive directional selection on antibacterial activity and stabilizing selection on PO-like activity. This finding is interesting, suggesting that the activity of different components of the snail immune system may be independently subjected to selection owing to differences in their importance for snails defences under certain conditions and/or trade-offs with other traits that are relevant for fitness. In this case, for instance, contrasting fitness functions may arise from possibly higher fitness costs of high PO-like activity that is a component of oxidative defences that potentially induce higher self-damage [108] than antibacterial activity. The variation in selection on the examined immune traits calls for simultaneous examination of a broader range of different immunological mechanisms.

To enable such work at the gene expression level, *L. stagnalis* has recently been subjected to extensive transcriptome sequencing [109]. That work has provided a broad picture of the immune system of this species and identified multiple targets for future ecoimmunological work. Transcriptomes were sequenced from individual snails exposed to various immune activation treatments (wounding, injection of bacteria cells, injection of trematode-infected snail tissue from other individuals) and environmental changes (elevated temperature, resource limitation). This approach allowed the identification of components of the immune system that respond to different immune challenges/environmental conditions. For instance, bacterial challenge activated the Toll-like receptor (TLR) signalling pathway, signalling through cytokines, antibacterial defences through cytolytic β pore-forming toxins and melanisation-type reaction [109]. Similarly, exposure to protein extracts from trematode parasites increased the gene expression of some components of the TLR signalling pathway and melanisation-type reaction. Additionally, apart from immune challenges, altered temperature and resource availability modified the expression levels of cytokines and effectors contributing to antibacterial defence [109]. These findings indicate a potentially important role of these components in the snail immune system against parasites and pathogens, as well as in determining context-dependence of immune activity.

However, by nature, many components of the invertebrate innate-type immune system show largely constant, unchanging levels of activity. Nevertheless, those components can be important determinants of the hosts' capacity to resist infections, thus contributing to organismal fitness. If such immunological mechanisms show high among-individual variation in natural populations, they could be subject to strong natural selection. Detecting variation in transcription that arises through causes such as genetic background and/or physiological condition of individuals is, however, easily overlooked in typical RNA-seq studies that aim to expose study organisms that are as genetically homogeneous as possible to highly controlled experimental treatments. To be able to detect such among-individual variation in immune activity, *L. stagnalis* transcriptomes [109] were specifically sequenced using a genetically diverse laboratory population of snails (see [106]). Interestingly, the results indicated high among-individual variation in the transcription of many components of the snail immune system, including non-self recognition, signalling through TLR pathway and cytokines, components of the production of reactive oxygen species (ROS), factors regulating apoptosis and effectors representing antibacterial defence and melanisation-type reaction [109]. In addition to immunological mechanisms that showed clear responses to immune challenges (see the

previous paragraph), immune factors with high among-individual variation in transcription should be included in future ecoimmunological studies on this species. For instance, cage experiments, similar to Langeloh *et al*. [106] that estimate snail fitness under (semi)natural conditions in the field, but employ targeted molecular assays (microarray or qRT-PCR) to quantify immune activity across a broad range of different immune defence factors at the transcription level would allow comprehensive examination of selection on snail immune phenotypes.

Earlier work examining the amount of within-population genetic variation in parasite resistance and immune activity in molluscs is slightly more abundant than the work on natural selection on defence traits that was described above. For example, Grosholz [16] examined genetic variation in the resistance of a bivalve mollusc *Transennella tantilla* against trematode parasites under field conditions. By maintaining individuals from laboratory cultured maternal sibships in field enclosures, he demonstrated significant family-level variation in parasite resistance. Similar variation has been seen in the susceptibility of *L. stagnalis* snails to trematode cercariae in laboratory exposures [110]. In *L. stagnalis*, family-level variation in immune activity (antibacterial activity and PO-like activity of haemolymph) has also been demonstrated under laboratory conditions using both maternal sibships [80,111] and full-sib families [112,113]. Although the conducted studies demonstrate the role of within-population genetic variation in determining susceptibility to infections and the strength of the immune defence, the fact that they are limited to comparisons among maternal sibships and full-sib families prevents their use in disentangling the actual genetic mechanisms that determine variation (e.g. additive versus dominance variance) and means that the results can be confounded by parental effects (but see [112]). Therefore, the studies conducted on molluscs cannot estimate the evolutionary potential of the immune defence traits/parasite resistance based on narrow-sense heritability that is defined by breeding values.

Recent and ongoing work on the genomics of *L. stagnalis* may provide great opportunities to use the tools of molecular quantitative genetics when examining variation in immune activity in natural snail populations under field conditions. Currently, a draft genome of *L. stagnalis* is available [114], and this species has been successfully used in a RAD-seq study to identify the chirality-determining locus in which the restriction enzyme SbfI produced 52 124 candidate loci [115]. This study, however, used paired-end sequencing and did not report how many of the candidate loci are located in physical proximity. Strong linkage between loci could significantly reduce the number of independent markers that can be used when building a genomic relatedness matrix. Nevertheless, the obtained number of loci should generate a sufficient marker density considering the genome size of 1.19 Gb of *L. stagnalis* [116] for molecular quantitative genetic analyses (i.e. estimation of trait heritability, chromosome partitioning analysis). The number of polymorphic marker loci provided by RAD-seq may, however, vary among snail populations depending on their genetic polymorphism. For example, preliminary results from a study of *L. stagnalis* populations in northern Switzerland that used the same SbfI enzyme with single-end sequencing recovered 7407 marker loci, many without any polymorphism, so that the number of polymorphic sites varied between 1456 and 2689 per population (C Çetin, PGD Feulner, O Seppälä 2020, personal observations). This result calls for the use of a more flexible double-digest RAD-seq approach in which different combinations of restriction enzymes are used to yield a greater number of markers [91].

5. Opportunities and challenges in ecoimmunology across molluscan gastropods

The scope of previous work on natural selection on and the evolutionary potential of immune defence traits in molluscs is narrow due to reliance on *L. stagnalis*. Also, the development of 'omics' resources (including annotation and expression profiling of immune genes) for this species is recent and still partly underway [109]. The increasing use of next-generation sequencing has begun to unlock other gastropod species as potential targets for ecoimmunological research by providing useful, and in some cases, well-developed genomics resources [117]. From the angle of gastropod immunogenomics, *Biomphalaria glabrata* is the most intensively studied species with a relatively well-annotated reference genome [37]. However, research on *B. glabrata* mainly focuses on understanding the molecular mechanisms that determine its, and other *Biomphalaria* species [118], resistance/susceptibility to *Schistosoma mansoni*, a trematode parasite that is a global human health problem [119]. The 'omics'-level work on the immune function of *B. glabrata* [120] has revealed commonalities of the general molluscan defence system when compared to other taxa. These include, for instance, the roles of lectins in non-self recognition, TLR signalling for immune regulation, and antimicrobial proteins and ROS production by haemocytes to eliminate pathogens. Although lineage-specific differences occur, for example, between prosobranch and heterobranch snails and even between closely related families like Planorbidae and Physidae [121], work on *B. glabrata* provides a useful resource to support ecoimmunological studies in other taxa. Research on *B. glabrata* also aims to identify targets in snail biology that may help to develop control measures of this species in nature to reduce human exposure to schistosomes. That effort logically calls for combining molecular immunology with field ecology and requires ecoimmunological investigations.

The New Zealand mud snail, *Potamopyrgus antipodarum*, is another good candidate for studies combining immunogenomics and ecology in gastropods. Longstanding studies on this species as a model for the evolutionary maintenance of sexual reproduction have motivated intensive examination of its transcriptomes, with a strong focus to characterize the immune system [39,122]. With a well-established understanding of the ecology of this species, *P. antipodarum* offers an excellent opportunity for combining field ecology and immunogenomics to extend the use of this model beyond the current focus on maintenance of sex. Furthermore, the development and expansion of genomics resources render additional gastropod species as potential candidates for ecoimmunological research. This includes, for example, the periwinkle *Littorina littorina*, whose immune system is extensively characterized (e.g. [123,124]), and *Physella acuta*, a freshwater snail for which current resources include a draft genome assembly and RNA-seq-based characterization of immunity [125]. Therefore, we believe that the opportunities of merging immunogenomics with ecological research can provide exciting new insights into the evolution of immune function across multiple gastropod species.

Results considering the variation in immune activity, its genetic basis and fitness consequences need, however, to be interpreted cautiously, especially when the examined immunological mechanisms are inducible. For example, in the most commonly used ecoimmunological model species *L. stagnalis*, both phenotypic immunological assays [126] and transcriptome data [109] indicate increased immune activity after an immune challenge in certain components of defence. Furthermore, environmental conditions such as food availability and temperature influence snails' immune function (e.g. [80,109,111]). Such effects may lead to temporal variation in immune activity at an individual level, which can hinder detecting the quantitative genetic basis and/or fitness consequences of among-individual variation in immune function when, for example, field-collected individuals are used. Therefore, the infection status (e.g. trematode infections) and resource level (e.g. fat content) of snails should be examined simultaneously with their immune activity if possible. Examining exposure to all relevant parasite types is, however, unrealistic in most studies. Furthermore, detecting parasite exposures that did not lead to an infection but that activated the immune system are virtually impossible to quantify. Therefore, the components of the innate-type immune system of molluscs that show largely constant levels of activity may be the most suitable for the evolutionary analyses suggested in this article. Transcriptome profiling of *L. stagnalis* has revealed multiple immunological mechanisms with high among-individual variation without indication of responses to immune activation or environmental factors (e.g. components of non-self recognition, TLR signalling, ROS production, antibacterial activity [109]). Those mechanisms serve as promising candidates for future research. Similar opportunities can be expected in other invertebrates that lack the adaptive immunity of vertebrates with the highest potential for induced responses.

6. Conclusion

While biomedical science has successfully eliminated several disease-causing agents (mostly viruses and bacteria), parasites are still one of the most common causes of death in humans and crop species, thus causing severe economic losses (e.g. [8,9]). Furthermore, the continuous emergence of new disease-causing agents [10,11], the evolution of drug resistance (reviewed in [12,13]) and biological invasions (reviewed in [14]) increase the disease risk now and in the future. Several molluscs transmit harmul parasites such as the human blood fluke (*S. mansoni*) in tropical regions [119,127], and liver fluke (*Fasciola hepatica*), fish eye flukes (*Diplostomum* spp.) and bird schistosomes (*Trichobilharzia* spp.) that cause swimmer's itch in temperate regions (e.g. [128–130]). Therefore, an essential element when creating projections of disease risks is to understand if and how natural host populations may evolutionarily adapt to parasitism.

Adaptive evolution of quantitative traits such as many components of parasite resistance and immune function requires that traits are subject to selection (i.e. contribute to organismal fitness) and that they are heritable (i.e. show additive genetic variance; [36]). Despite broad interest in the evolutionary ecology of immune activity in animals, the understanding of selection on and evolutionary potential of immune defence traits is not comprehensive. For example, empirical studies typically do not support theoretical predictions of immune activity being subject to stabilizing selection (reviewed in [46]). We propose that this discrepancy may be because ecoimmunological studies that mostly examine one/few immunological mechanisms cover only a fraction of the complexity of an animal immune system. The same mostly holds for molecular immunology/immunogenetics studies that also neglect variation in immune activity that arises from genetic variation among individuals and from environmental conditions. We believe that 'merging' ecological immunology, genomics and transcriptomics is necessary to fill these knowledge gaps and combine the formerly separated field of ecological and molecular/genetic immunology. We see this approach as highly promising in various taxa of molluscan gastropods that are already used as model systems in ecological and evolutionary research (e.g. *L. stagnalis*, *P. antipodarum*), molecular immunology (e.g. *B. glabrata*, *L. stagnalis*) and genomics (e.g. *B. glabrata*). Combining the knowledge and tools across the disiplines in these model species should allow examination of evolution of immune activity while simultaneously covering the immune system as a whole and considering the ecologically relevant genetic background and environmental conditions. Only then can evolutionary processes in natural populations be thoroughly estimated.

Data accessibility. No data were used in this article.

Authors' contributions. O.S. developed the concept for the manuscript and wrote the first draft. C.Ç., T.C., P.G.D.F. and C.M.A. contributed to the development of the ideas and the text. All authors gave final approval for publication.

Competing interests. No competing interests.

Funding. This work was supported by the Swiss National Science Foundation (grant no. 31003A 169531) and ETH research commission (grant no. ETH-20 17-1) to O.S.

Acknowledgements. We thank M. Neiman, A. Davison and the Royal Society for organizing and hosting the meeting 'Pearls of wisdom: synergising leadership and expertize in molluscan genomics' as well as for the invitation to contribute to this Theo Murphy issue. We are grateful to anonymous reviewers for commenting on the manuscript.

References

1. Zuk M, Stoehr AM. 2002 Immune defense and host life history. *Am. Nat.* **160**, S9–S22. (doi:10.1086/342131)
2. Nunn CL, Lindenfors P, Pursall ER, Rolff J. 2009 On sexual dimorphism in immune function. *Phil. Trans. R. Soc. B* **364**, 61–69. (doi:10.1098/rstb.2008.0148)
3. Hamilton WD, Zuk M. 1982 Heritable true fitness and bright birds: a role for parasites. *Science* **218**, 384–387. (doi:10.1126/science.7123238)
4. Rantala MJ, Koskimaki J, Taskinen J, Tynkkynen K, Suhonen J. 2000 Immunocompetence, developmental stability and wingspot size in the damselfly *Calopteryx splendens* L. *Proc. R. Soc. B* **267**, 2453–2457. (doi:10.1098/rspb.2000.1305)
5. Tompkins DM, Begon M. 1999 Parasites can regulate wildlife populations. *Parasitol. Today* **15**, 311–313. (doi:10.1016/S0169-4758(99)01484-2)
6. Fürst MA, McMahon DP, Osborne JL, Paxton RJ, Brown MJF. 2014 Disease associations between

honeybees and bumblebees as a threat to wild pollinators. *Nature* **506**, 364–366. (doi:10.1038/nature12977)

7. Martin SJ, Highfield AC, Brettell L, Villalobos EM, Budge GE, Powell M, Nikaido S, Schroeder DC. 2012 Global honey bee viral landscape altered by a parasitic mite. *Science* **336**, 1304–1306. (doi:10.1126/science.1220941)
8. Roberts T, Murrell KD, Marks S. 1994 Economic-losses caused by foodborne parasitic diseases. *Parasitol. Today* **10**, 419–423. (doi:10.1016/0169-4758(94)90171-6)
9. Perry BD, Randolph TF. 1999 Improving the assessment of the economic impact of parasitic diseases and of their control in production animals. *Vet. Parasitol.* **84**, 145–168. (doi:10.1016/S0304-4017(99)00040-0)
10. Antia R, Regoes RR, Koella JC, Bergstrom CT. 2003 The role of evolution in the emergence of infectious diseases. *Nature* **426**, 658–661. (doi:10.1038/nature02104)
11. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P. 2008 Global trends in emerging infectious diseases. *Nature* **451**, 990–993. (doi:10.1038/nature06536)
12. Anderson JB. 2005 Evolution of antifungal-drug resistance: mechanisms and pathogen fitness. *Nat. Rev. Microbiol.* **3**, 547–556. (doi:10.1038/nrmicro1179)
13. Read AF, Day T, Huijben S. 2011 The evolution of drug resistance and the curious orthodoxy of aggressive chemotherapy. *Proc. Natl Acad. Sci. USA* **108**, 10 871–10 877. (doi:10.1073/pnas.1100299108)
14. Dunn AM, Hatcher MJ. 2015 Parasites and biological invasions: parallels, interactions, and control. *Trends Parasitol.* **31**, 189–199. (doi:10.1016/j.pt.2014.12.003)
15. Richards CS, Knight M, Lewis FA. 1992 Genetics of *Biomphalaria glabrata* and its effect on the outcome of *Schistosoma mansoni* infection. *Parasitol. Today* **8**, 171–174. (doi:10.1016/0169-4758(92)90015-T)
16. Grosholz ED. 1994 The effects of host genotype and spatial distribution on trematode parasitism in a bivalve population. *Evolution* **48**, 1514–1524. (doi:10.1111/j.1558-5646.1994.tb02193.x)
17. Koskela T, Puustinen S, Salonen V, Mutikainen P. 2002 Resistance and tolerance in a host plant–holoparasitic plant interaction: genetic variation and costs. *Evolution* **56**, 899–908. (doi:10.1111/j.0014-3820.2002.tb01403.x)
18. Love OP, Salvante KG, Dale J, Williams TD. 2008 Sex-specific variability in the immune system across life-history stages. *Am. Nat.* **172**, E99–E112. (doi:10.1086/589521)
19. Hayward AD, Wilson AJ, Pilkington JG, Pemberton JM, Kruuk LEB. 2009 Ageing in a variable habitat: environmental stress affects senescence in parasite resistance in St Kilda Soay sheep. *Proc. R. Soc. B* **276**, 3477–3485. (doi:10.1098/rspb.2009.0906)
20. Palacios MG, Winkler DW, Klasing KC, Hasselquist D, Vleck CM. 2011 Consequences of immune system aging in nature: a study of immunosenescence costs in free-living tree swallows. *Ecology* **92**, 952–966. (doi:10.1890/10-0662.1)
21. Murray DL, Keith LB, Cary JR. 1998 Do parasitism and nutritional status interact to affect production in snowshoe hares? *Ecology* **79**, 1209–1222. (doi:10.1890/0012-9658(1998)079[1209:DPANSI]2.0.CO;2)
22. Kolluru GR, Grether GF, South SH, Dunlop E, Cardinali A, Liu L, Carapiet A. 2006 The effects of carotenoid and food availability on resistance to a naturally occurring parasite (*Gyrodactylus turnbulli*) in guppies (*Poecilia reticulata*). *Biol. J. Linn. Soc.* **89**, 301–309. (doi:10.1111/j.1095-8312.2006.00675.x)
23. Hutchings MR, Gordon IJ, Kyriazakis I, Jackson F. 2001 Sheep avoidance of faeces-contaminated patches leads to a trade-off between intake rate of forage and parasitism in subsequent foraging decisions. *Anim. Behav.* **62**, 955–964. (doi:10.1006/anbe.2001.1837)
24. Hall SR, Sivars-Becker L, Becker C, Duffy MA, Tessier AJ, Cáceres CE. 2007 Eating yourself sick: transmission of disease as a function of foraging ecology. *Ecol. Lett.* **10**, 207–218. (doi:10.1111/j.1461-0248.2007.01011.x)
25. Wilson K, Thomas MB, Blanford S, Doggett M, Simpson SJ, Moore SL. 2002 Coping with crowds: density-dependent disease resistance in desert locusts. *Proc. Natl Acad. Sci. USA* **99**, 5471–5475. (doi:10.1073/pnas.082461999)
26. Mitchell SE, Rogers ES, Little TJ, Read AF. 2005 Host–parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterilising pathogen. *Evolution* **59**, 70–80. (doi:10.1111/j.0014-3820.2005.tb00895.x)
27. Janeway CA, Travers P, Walport M, Shlomchik M. 2005 *Immunobiology: the immune system in health and disease*. New York, NY: Garland Science.
28. Schmid-Hempel P. 2011 *Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics*. New York, NY: Oxford University Press.
29. Demas GE, Nelson RJ. 2012 *Ecoimmunology*. New York, NY: Oxford University Press.
30. Hawley DM, Altizer SM. 2011 Disease ecology meets ecological immunology: understanding the links between organismal immunity and infection dynamics in natural populations. *Funct. Ecol.* **25**, 48–60. (doi:10.1111/j.1365-2435.2010.01753.x)
31. Moret Y. 2003 Explaining variable costs of the immune response: selection for specific versus non-specific immunity and facultative life history change. *Oikos* **102**, 213–216. (doi:10.1034/j.1600-0706.2003.12496.x)
32. Cotter SC, Kruuk LEB, Wilson K. 2004 Costs of resistance: genetic correlations and potential trade-offs in an insect immune system. *J. Evol. Biol.* **17**, 421–429. (doi:10.1046/j.1420-9101.2003.00655.x)
33. Rolff J, Armitage SAO, Coltman DW. 2005 Genetic constraints and sexual dimorphism in immune defense. *Evolution* **59**, 1844–1850. (doi:10.1111/j.0014-3820.2005.tb01831.x)
34. Schwarzenbach GA, Hosken DJ, Ward PI. 2005 Sex and immunity in the yellow dung fly *Scathophaga stercoraria*. *J. Evol. Biol.* **18**, 455–463. (doi:10.1111/j.1420-9101.2004.00820.x)
35. Lively CM. 2001 Parasite-host interactions. In *Evolutionary ecology: concepts and case studies* (eds CW Fox, DA Roff, DJ Fairbairn), pp. 290–302. Oxford, UK: Oxford University Press.
36. Endler JA. 1986 *Natural selection in the wild*. Princeton, NJ: Princeton University Press.
37. Adema CM *et al.* 2017 Whole genome analysis of a schistosomiasis-transmitting freshwater snail. *Nat. Commun.* **8**, 1–2. (doi:10.1038/ncomms15451)
38. Deleury E *et al.* 2012 Specific versus non-specific immune responses in an invertebrate species evidenced by a comparative *de novo* sequencing study. *PLoS ONE* **7**, e32512. (doi:10.1371/journal.pone.0032512)
39. Bankers L, Fields P, McElroy KE, Boore JL, Logsdon JM, Neiman M. 2017 Genomic evidence for population-specific responses to co-evolving parasites in a New Zealand freshwater snail. *Mol. Ecol.* **26**, 3663–3675. (doi:10.1111/mec.14146)
40. Lindström KM, Foufopoulos J, Pärn H, Wikelski M. 2004 Immunological investments reflect parasite abundance in island populations of Darwin's finches. *Proc. R. Soc. B* **271**, 1513–1519. (doi:10.1098/rspb.2004.2752)
41. Scharsack JP, Kalbe M, Harrod C, Rauch G. 2007 Habitat-specific adaptation of immune responses of stickleback (*Gasterosteus aculeatus*) lake and river ecotypes. *Proc. R. Soc. B* **274**, 1523–1532. (doi:10.1098/rspb.2007.0210)
42. Lochmiller RL, Deerenberg C. 2000 Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88**, 87–98. (doi:10.1034/j.1600-0706.2000.880110.x)
43. Demas G, Greives T, Chester E, French S. 2012 The energetics of immunity: mechanisms mediating trade-offs in ecoimmunology. In *Ecoimmunology* (eds GE Demas, RJ Nelson), pp. 259–296. New York, NY: Oxford University Press.
44. Ilmonen P, Taarna T, Hasselquist D. 2000 Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. *Proc. R. Soc. B* **267**, 665–670. (doi:10.1098/rspb.2000.1053)
45. Moret Y, Schmid-Hempel P. 2001 Immune defence in bumble-bee offspring. *Nature* **414**, 506. (doi:10.1038/35107138)
46. Seppälä O. 2015 Natural selection on quantitative immune defence traits: a comparison between theory and data. *J. Evol. Biol.* **28**, 1–9. (doi:10.1111/jeb.12528)
47. Svensson E, Sinervo B, Comendant T. 2001 Density-dependent competition and selection on immune function in genetic lizard morphs. *Proc. Natl Acad. Sci. USA* **98**, 12 561–12 565. (doi:10.1073/pnas.211071298)
48. Råberg L, Stjernman M. 2003 Natural selection on immune responsiveness in blue tits *Parus caeruleus*. *Evolution* **57**, 1670–1678. (doi:10.1554/02-417)
49. Calsbeek R, Bonneaud C, Smith TB. 2008 Differential fitness effects of immunocompetence and neighbourhood density in alternative female lizard morphs. *J. Anim. Ecol.* **77**, 103–109. (doi:10.1111/j.1365-2656.2007.01320.x)

50. Graham AL, Hayward AD, Watt KA, Pilkington JG, Pemberton JM, Nussey DH. 2010 Fitness correlates of heritable variation in antibody responsiveness in a wild mammal. *Science* **330**, 662–665. (doi:10.1126/science.1194878)
51. Watson RL *et al.* 2016 Cellular and humoral immunity in a wild mammal: variation with age & sex and association with overwinter survival. *Ecol. Evol.* **6**, 8695–8705. (doi:10.1002/ece3.2584)
52. Ghosh J, Lun CM, Majeske AJ, Sacchi S, Schrankel CS, Smith LC. 2011 Invertebrate immune diversity. *Dev. Comp. Immunol.* **35**, 959–974. (doi:10.1016/j.dci.2010.12.009)
53. Royet J, Reichhart JM, Hoffmann JA. 2005 Sensing and signaling during infection in *Drosophila*. *Curr. Opin. Immunol.* **17**, 11–17. (doi:10.1016/j.coi.2004.12.002)
54. Hetru C, Hoffmann JA. 2009 NF-kappa B in the immune response of *Drosophila*. *Cold Spring Harb. Perspect. Biol.* **1**, a000232. (doi:10.1101/cshperspect.a000232)
55. Doublet V *et al.* 2017 Unity in defence: honeybee workers exhibit conserved molecular responses to diverse pathogens. *BMC Genomics* **18**, 1–7. (doi:10.1186/s12864-017-3624-7)
56. Hanelt B, Lun CM, Adema CM. 2008 Comparative ORESTES-sampling of transcriptomes of immune-challenged *Biomphalaria glabrata* snails. *J. Invertebr. Pathol.* **99**, 192–203. (doi:10.1016/j.jip.2008.06.002)
57. Adema CM, Hanington PC, Lun CM, Rosenberg GH, Aragon AD, Stout BA, Lennard Richard ML, Gross PS, Loker ES. 2010 Differential transcriptomic responses of *Biomphalaria glabrata* (Gastropoda, Mollusca) to bacteria and metazoan parasites, *Schistosoma mansoni* and *Echinostoma paraensei* (Digenea, Platyhelminthes). *Mol. Immunol.* **47**, 849–860. (doi:10.1016/j.molimm.2009.10.019)
58. Wang Z, Gerstein M, Snyder M. 2009 RNA-Seq: a revolutionary tool for transcriptomics. *Nat. Rev. Genet.* **10**, 57–63. (doi:10.1038/nrg2484)
59. Rapaport F, Khanin R, Liang YP, Pirun M, Krek A, Zumbo P, Mason CE, Socci ND, Betel D. 2013 Comprehensive evaluation of differential gene expression analysis methods for RNA-seq data. *Genome Biol.* **14**, R95. (doi:10.1186/gb-2013-14-9-r95)
60. Enard W *et al.* 2002 Intra- and interspecific variation in primate gene expression patterns. *Science* **296**, 340–343. (doi:10.1126/science.1068996)
61. Gilad Y, Oshlack A, Smyth GK, Speed TP, White KP. 2006 Expression profiling in primates reveals a rapid evolution of human transcription factors. *Nature* **440**, 242–245. (doi:10.1038/nature04559)
62. Ferea TL, Botstein D, Brown PO, Rosenzweig RF. 1999 Systematic changes in gene expression patterns following adaptive evolution in yeast. *Proc. Natl Acad. Sci. USA* **96**, 9721–9726. (doi:10.1073/pnas.96.17.9721)
63. Brem RB, Yvert G, Clinton R, Kruglyak L. 2002 Genetic dissection of transcriptional regulation in budding yeast. *Science* **296**, 752–755. (doi:10.1126/science.1069516)
64. Jin W, Riley RM, Wolfinger RD, White KP, Passador-Gurgel G, Gibson G. 2001 The contributions of sex, genotype and age to transcriptional variance in *Drosophila melanogaster*. *Nat. Genet.* **29**, 389–395. (doi:10.1038/ng766)
65. Gibson G, Riley-Berger R, Harshman L, Kopp A, Vacha S, Nuzhdin S, Wayne M. 2004 Extensive sex-specific nonadditivity of gene expression in *Drosophila melanogaster*. *Genetics* **167**, 1791–1799. (doi:10.1534/genetics.104.026583)
66. Whitehead A, Crawford DL. 2006 Neutral and adaptive variation in gene expression. *Proc. Natl Acad. Sci. USA* **103**, 5425–5430. (doi:10.1073/pnas.0507648103)
67. Leder EH, McCairns RJS, Leinonen T, Cano JM, Viitaniemi HM, Nikinmaa M, Primmer CR, Merilä J. 2015 The evolution and adaptive potential of transcriptional variation in sticklebacks—signatures of selection and widespread heritability. *Mol. Biol. Evol.* **32**, 674–689. (doi:10.1093/molbev/msu328)
68. Gibson G, Weir B. 2005 The quantitative genetics of transcription. *Trends Genet.* **21**, 616–623. (doi:10.1016/j.tig.2005.08.010)
69. Dheilly NM, Adema C, Raftos DA, Gourbal B, Grunau C, Du Pasquier L. 2014 No more non-model species: the promise of next generation sequencing for comparative immunology. *Dev. Comp. Immunol.* **45**, 56–66. (doi:10.1016/j.dci.2014.01.022)
70. Pedersen AB, Babayan SA. 2011 Wild immunology. *Mol. Ecol.* **20**, 872–880. (doi:10.1111/j.1365-294X.2010.04938.x)
71. Brunner FS, Schmid-Hempel P, Barribeau SM. 2014 Protein-poor diet reduces host-specific immune gene expression in *Bombus terrestris*. *Proc. R. Soc. B* **281**, 20140128. (doi:10.1098/rspb.2014.0128)
72. Barribeau SM, Sadd B, du Plessis L, Schmid-Hempel P. 2014 Gene expression differences underlying genotype-by-genotype specificity in a host–parasite system. *Proc. Natl Acad. Sci. USA* **111**, 3496–3501. (doi:10.1073/pnas.1318628111)
73. Ferro K, Ferro D, Corrà F, Bakiu R, Santovito G, Kurtz J. 2017 Cu,Zn superoxide dismutase genes in *Tribolium castaneum*: evolution, molecular characterisation, and gene expression during immune priming. *Front. Immunol.* **8**, 1811. (doi:10.3389/fimmu.2017.01811)
74. Greenwood JM, Milutinović B, Peuß R, Behrens S, Esser D, Rosenstiel P, Schulenburg H, Kurtz J. 2017 Oral immune priming with *Bacillus thuringiensis* induces a shift in the gene expression of *Tribolium castaneum* larvae. *BMC Genomics* **18**, 1–4. (doi:10.1186/s12864-017-3705-7)
75. Teplitsky C, Robinson MR, Merilä J. 2014 Evolutionary potential and constraints in wild populations. In *Quantitative genetics in the wild* (eds A Charmantier, D Garant, LEB Kruuk), pp. 190–208. Oxford, UK: Oxford University Press. (doi:10.1093/acprof:oso/9780199674237.003.0012)
76. Bijma P, Wade MJ. 2008 The joint effects of kin, multilevel selection and indirect genetic effects on response to genetic selection. *J. Evol. Biol.* **21**, 1175–1188. (doi:10.1111/j.1420-9101.2008.01550.x)
77. Kruuk LEB, Slate J, Wilson AJ. 2008 New answers for old questions: the evolutionary quantitative genetics of wild animal populations. *Annu. Rev. Ecol. Evol. Syst.* **39**, 525–548. (doi:10.1146/annurev.ecolsys.39.110707.173542)
78. Larsson K, van der Jeugd HP, van der Veen IT, Forslund P. 1998 Body size declines despite positive directional selection on heritable size traits in a barnacle goose population. *Evolution* **52**, 1169–1184. (doi:10.1111/j.1558-5646.1998.tb01843.x)
79. Merilä J, Sheldon BC, Kruuk LEB. 2001 Explaining stasis: microevolutionary studies in natural populations. *Genetica* **112**, 199–222. (doi:10.1023/A:1013391806317)
80. Seppälä O, Jokela J. 2010 Maintenance of genetic variation in immune defense of a freshwater snail: role of environmental heterogeneity. *Evolution* **64**, 2397–2407. (doi:10.1111/j.1558-5646.2010.00995.x)
81. Pitala N, Gustafsson L, Sendecka J, Brommer JE. 2007 Nestling immune response to phytohaemagglutinin is not heritable in collared flycatchers. *Biol. Lett.* **3**, 418–421. (doi:10.1098/rsbl.2007.0135)
82. Kim SY, Fargallo JA, Vergara P, Martínez-Padilla J. 2013 Multivariate heredity of melanin-based coloration, body mass and immunity. *Heredity* **111**, 139–146. (doi:10.1038/hdy.2013.29)
83. Sakaluk SK, Wilson AJ, Bowers EK, Johnson LS, Masters BS, Johnson BGP, Vogel LA, Forsman AM, Thompson CF. 2014 Genetic and environmental variation in condition, cutaneous immunity, and haematocrit in house wrens. *BMC Evol. Biol.* **14**, 242. (doi:10.1186/s12862-014-0242-8)
84. Sparks AM, Watt K, Sinclair R, Pilkington JG, Pemberton JM, McNeilly TN, Nussey DH, Johnston SE. 2019 The genetic architecture of helminth-specific immune responses in a wild population of Soay sheep (*Ovis aries*). *PLoS Genet.* **15**, e1008461. (doi:10.1371/journal.pgen.1008461)
85. Reiner SL, Locksley RM. 1995 The regulation of immunity to *Leishmania major*. *Annu. Rev. Immunol.* **13**, 151–177. (doi:10.1146/annurev.iy.13.040195.001055)
86. Le Goff L, Lamb TJ, Graham AL, Harcus Y, Allen JE. 2002 IL-4 is required to prevent filarial nematode development in resistant but not susceptible strains of mice. *Int. J. Parasitol.* **32**, 1277–1284. (doi:10.1016/S0020-7519(02)00125-X)
87. Hoffmann AA, Merilä J. 1999 Heritable variation and evolution under favourable and unfavourable conditions. *Trends Ecol. Evol.* **14**, 96–101. (doi:10.1016/S0169-5347(99)01595-5)
88. Sgrò CM, Hoffmann AA. 2004 Genetic correlations, trade-offs and environmental variation. *Heredity* **93**, 241–248. (doi:10.1038/sj.hdy.6800532)
89. Postma E. 2014 Four decades of estimating heritabilities in wild vertebrate populations: improved methods, more data, better estimates? *Quant. Genet. Wild* **16**, 33. (doi:10.1093/acprof:oso/9780199674237.003.0002)
90. Davey JL, Blaxter MW. 2010 RADSeq: next-generation population genetics. *Brief. Funct. Genom.* **9**, 416–423. (doi:10.1093/bfgp/elq031)

91. Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. 2012 Double digest RADseq: an inexpensive method for *de novo* SNP discovery and genotyping in model and non-model species. *PLoS ONE* **7**, e37135. (doi:10.1371/journal.pone.0037135)
92. Powell JE, Visscher PM, Goddard ME. 2010 Reconciling the analysis of IBD and IBS in complex trait studies. *Nat. Rev. Genet.* **11**, 800–805. (doi:10.1038/nrg2865)
93. Robinson MR, Santure AW, DeCauwer I, Sheldon BC, Slate J. 2013 Partitioning of genetic variation across the genome using multimarker methods in a wild bird population. *Mol. Ecol.* **22**, 3963–3980. (doi:10.1111/mec.12375)
94. Santure AW, De Cauwer I, Robinson MR, Poissant J, Sheldon BC, Slate J. 2013 Genomic dissection of variation in clutch size and egg mass in a wild great tit (*Parus major*) population. *Mol. Ecol.* **22**, 3949–3962. (doi:10.1111/mec.12376)
95. Bérénos C, Ellis PA, Pilkington JG, Pemberton JM. 2014 Estimating quantitative genetic parameters in wild populations: a comparison of pedigree and genomic approaches. *Mol. Ecol.* **23**, 3434–3451. (doi:10.1111/mec.12827)
96. Bérénos C, Ellis PA, Pilkington JG, Lee SH, Gratten J, Pemberton JM. 2015 Heterogeneity of genetic architecture of body size traits in a free-living population. *Mol. Ecol.* **24**, 1810–1830. (doi:10.1111/mec.13146)
97. Lee SH, Goddard ME, Visscher PM, van der Werf JHJ. 2010 Using the realised relationship matrix to disentangle confounding factors for the estimation of genetic variance components of complex traits. *Genet. Sel. Evol.* **42**, 22. (doi:10.1186/1297-9686-42-22)
98. Wang JL. 2016 Pedigrees or markers: which are better in estimating relatedness and inbreeding coefficient? *Theor. Popul. Biol.* **107**, 4–13. (doi:10.1016/j.tpb.2015.08.006)
99. Gervais L, Perrier C, Bernard M, Merlet J, Pemberton JM, Pujol B, Quéméré E. 2019 RAD-sequencing for estimating genomic relatedness matrix-based heritability in the wild: a case study in roe deer. *Mol. Ecol. Resour.* **19**, 1205–1217. (doi:10.1111/1755-0998.13031)
100. Yang J, Zeng J, Goddard ME, Wray NR, Visscher PM. 2017 Concepts, estimation and interpretation of SNP-based heritability. *Nat. Genet.* **49**, 1304–1320. (doi:10.1038/ng.3941)
101. Yang J *et al.* 2011 Genome partitioning of genetic variation for complex traits using common SNPs. *Nat. Genet.* **43**, 519–525. (doi:10.1038/ng.823)
102. Hirschhorn JN, Daly MJ. 2005 Genome-wide association studies for common diseases and complex traits. *Nat. Rev. Genet.* **6**, 95–108. (doi:10.1038/nrg1521)
103. Howick VM, Lazzaro BP. 2017 The genetic architecture of defence as resistance to and tolerance of bacterial infection in *Drosophila melanogaster*. *Mol. Ecol.* **26**, 1533–1546. (doi:10.1111/mec.14017)
104. Fournier-Level A, Korte A, Cooper MD, Nordborg M, Schmitt J, Wilczek AM. 2011 A map of local adaptation in *Arabidopsis thaliana*. *Science* **334**, 86–89. (doi:10.1126/science.1209271)
105. Barson NJ *et al.* 2015 Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. *Nature* **528**, 405–408. (doi:10.1038/nature16062)
106. Langeloh L, Behrmann-Godel J, Seppälä O. 2017 Natural selection on immune defense: a field experiment. *Evolution* **71**, 227–237. (doi:10.1111/evo.13148)
107. Kopp KC, Wolff K, Jokela J. 2012 Natural range expansion and human-assisted introduction leave different genetic signatures in a hermaphroditic freshwater snail. *Evol. Ecol.* **26**, 483–498. (doi:10.1007/s10682-011-9504-8)
108. Sadd BM, Siva-Jothy MT. 2006 Self-harm caused by an insect's innate immunity. *Proc. R. Soc. B* **273**, 2571–2574. (doi:10.1098/rspb.2006.3574)
109. Seppälä O, Walser J-C, Cereghetti T, Seppälä K, Salo T, Adema CM. 2021 Transcriptome profiling of *Lymnaea stagnalis* (Gastropoda) for ecoimmunological research. *BMC Genomics*. **22**, 44. (doi:10.1186/s12864-021-07428-1)
110. Seppälä O, Karvonen A, Haataja M, Kuosa M, Jokela J. 2011 Food makes you a target: disentangling genetic, physiological, and behavioral effects determining susceptibility to infection. *Evolution* **65**, 1367–1375. (doi:10.1111/j.1558-5646.2010.01205.x)
111. Leicht K, Seppälä K, Seppälä O. 2017 Potential for adaptation to climate change: family-level variation in fitness-related traits and their responses to heat waves in a snail population. *BMC Evol. Biol.* **17**, 140. (doi:10.1186/s12862-017-0988-x)
112. Seppälä O, Langeloh L. 2016 Estimating genetic and maternal effects determining variation in immune function of a mixed-mating snail. *PLoS ONE* **10**, e0161584. (doi:10.1371/journal.pone.0161584)
113. Leicht K, Jokela J, Seppälä O. 2019 Inbreeding does not alter the response to an experimental heat wave in a freshwater snail. *PLoS ONE* **14**, e0220669. (doi:10.1371/journal.pone.0220669)
114. Davison A *et al.* 2016 Formin is associated with left-right asymmetry in the pond snail and the frog. *Curr. Biol.* **26**, 654–660. (doi:10.1016/j.cub.2015.12.071)
115. Liu MM, Davey JW, Banerjee R, Han J, Yang F, Aboobaker A, Blaxter ML, Davison A. 2013 Fine mapping of the pond snail left-right asymmetry (chirality) locus using RAD-Seq and Fibre-FISH. *PLoS ONE* **8**, e71067. (doi:10.1371/journal.pone.0071067)
116. Vinogradov AE. 1998 Variation in ligand-accessible genome size and its ecomorphological correlates in a pond snail. *Hereditas* **128**, 59–65. (doi:10.1111/j.1601-5223.1998.00059.x)
117. Schultz JH, Adema CM. 2017 Comparative immunogenomics of molluscs. *Dev. Comp. Immunol.* **75**, 3–15. (doi:10.1016/j.dci.2017.03.013)
118. Buddenborg SK, Bu LJ, Zhang S-M, Schilkey FD, Mkoji GM, Loker ES. 2017 Transcriptomic responses of *Biomphalaria pfeifferi* to *Schistosoma mansoni*: investigation of a neglected African snail that supports more *S. mansoni* transmission than any other snail species. *PLoS Negl. Trop. Dis.* **11**, e0005984. (doi:10.1371/journal.pntd.0005984)
119. GBD 2017. 2019 Disease and injury incidence and prevalence collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study (vol. 392, pg 1789, 2018). *Lancet* **393**, E44. (doi:10.1016/S0140-6736(19)31429-1)
120. Castillo MG, Humphries JE, Mourão MM, Marquez J, Gonzalez A, Montelongo CE. 2020 *Biomphalaria glabrata* immunity: post-genome advances. *Dev. Comp. Immunol.* **104**, 103557. (doi:10.1016/j.dci.2019.103557)
121. Schultz JH, Bu LJ, Adema CM. 2018 Comparative immunological study of the snail *Physella acuta* (Hygrophila, Pulmonata) reveals shared and unique aspects of gastropod immunobiology. *Mol. Immunol.* **101**, 108–119. (doi:10.1016/j.molimm.2018.05.029)
122. Wilton PR, Sloan DB, Logsdon JM, Doddapaneni H, Neiman M. 2013 Characterization of transcriptomes from sexual and asexual lineages of a New Zealand snail (*Potamopyrgus antipodarum*). *Mol. Ecol. Resour.* **13**, 289–294. (doi:10.1111/1755-0998.12051)
123. Gorbushin AM. 2019 Immune response of a caenogastropod host: a case study of *Littorina littorea* and its digenean parasites. *Dev. Comp. Immunol.* **101**, 103465. (doi:10.1016/j.dci.2019.103465)
124. Gorbushin AM, Borisova EA. 2015 Lectin-like molecules in transcriptome of *Littorina littorea* hemocytes. *Dev. Comp. Immunol.* **48**, 210–220. (doi:10.1016/j.dci.2014.10.007)
125. Schultz JH, Bu LJ, Kamel B, Adema CM. 2020 Rna-Seq: the early response of the snail *Physella acuta* to the digenetic trematode *Echinostoma paraensei*. *J. Parasitol.* **106**, 490–505. (doi:10.1645/19-36)
126. Seppälä O, Leicht K. 2013 Activation of the immune defence of the freshwater snail *Lymnaea stagnalis* by different immune elicitors. *J. Exp. Biol.* **216**, 2902–2907. (doi:10.1242/jeb.084947)
127. Deol AK *et al.* 2019 Schistosomiasis: assessing progress toward the 2020 and 2025 global goals. *N. Engl. J. Med.* **381**, 2519–2528. (doi:10.1056/NEJMoa1812165)
128. Rinaldi L *et al.* 2015 Sheep and *Fasciola hepatica* in Europe: the GLOWORM experience. *Geospatial Health* **9**, 309–317. (doi:10.4081/gh.2015.353)
129. Karvonen A, Savolainen M, Seppälä O, Valtonen ET. 2006 Dynamics of *Diplostomum spathaceum* infection in snail hosts at a fish farm. *Parasitol. Res.* **99**, 341–345. (doi:10.1007/s00436-006-0137-8)
130. Horák P, Mikeš L, Lichtenbergová L, Skala V, Soldánová M, Brant SV. 2015 Avian schistosomes and outbreaks of cercarial dermatitis. *Clin. Microbiol. Rev.* **28**, 165–190. (doi:10.1128/CMR.00043-14)