

**Examining adaptive evolution of immune activity: opportunities provided by gastropods
in the age of “omics”**

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Abstract

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 stabilising 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.

Keywords Gastropoda, heritability, immune function, immunocompetence, *Lymnaea stagnalis*.

1. Introduction

Parasites [here referring to both micro- (e.g., viruses and bacteria) and macro-parasites (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 jeopardise 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 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 [see e.g., (37-39)] could be of great help strengthening future ecoimmunological research.

2. Natural selection on immune activity

The first requirement for adaptive evolution of a phenotypic trait is that it is subject to natural selection. From the potential forms of selection on quantitative traits [see (36)], positive directional (i.e., the highest trait values lead to the highest fitness) and stabilising 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 stabilising 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 stabilising 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 [see (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 at 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 expression levels 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 [see (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 the 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 been conducted only 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 been conducted.

In the field of quantitative genetics, interest in using genomics tools when examining heritability of phenotypic traits is currently increasing. Utilising genomics methods allows, for instance, genotyping of individuals with high marker density across the whole genome [e.g., 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 realised 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 has proven to be highly useful by improving parameter estimates when compared with those that utilise only pedigree information (95, 97, 98). Additionally, RAD-seq data has 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 utilising 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 ($> 1\%$ 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 [see (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 stabilising selection aimed to be minimised [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 stabilising selection on PO-like activity. This finding is interesting by 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 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 gene expression 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 expression levels 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 gene expression 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 gene expression 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*, also family-level variation in immune activity (antibacterial activity and PO-like activity of haemolymph) has 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 vs. 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 defense 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 utilise 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, utilised 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 (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 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 characterise 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 immunity system is extensively characterised [e.g., (123, 124)] and *Physella acuta*, a freshwater snail for which current resources include a draft genome assembly and RNAseq-based characterisation 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 adaptive immunity of vertebrates with the highest potential for induced responses.

6. Conclusions

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 harmful 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) [see (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 stabilising 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 formerly separated field of ecological and molecular/genetic immunology. We see this approach 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 disciplines in these model species should allow examining evolution of immune activity while simultaneously covering the immune system as a whole and considering ecologically relevant genetic background and environmental conditions. Only then can evolutionary processes in natural populations be thoroughly estimated.

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