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Genomics of hosts-pathogen interactions: challenges and opportunities across ecological and spatiotemporal scales

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Evolutionary genomics has recently entered a new era in the study of host-pathogen interactions. A variety of novel genomic techniques has transformed to the identification, detection and classification of both hosts and pathogens, allowing a greater resolution that helps decipher their underlying dynamics and provides novel insights into their environmental context. Nevertheless, many challenges to a general understanding of host-pathogen interactions remain, in particular in the synthesis and integration of concepts and findings across a variety of systems and different spatiotemporal and ecological scales. In this perspective we aim to highlight some of the commonalities and complexities across diverse studies of host-pathogen interactions, with a focus on ecological, spatiotemporal variation, and the choice of genomic methods used. We performed a quantitative review of recent literature to investigate links, patterns and potential tradeoffs between the complexity of genomic, ecological and spatiotemporal scales undertaken in individual host-pathogen studies. We found that the majority of studies used whole genome resolution to address their research objectives across a broad range of ecological

scales, especially when focusing on the pathogen side of the interaction. Nevertheless, genomic studies conducted in a complex spatiotemporal context are currently rare in the literature. Because processes of host-pathogen interactions can be understood at multiple scales, from molecular-, cellular-, and physiological-scales to the levels of populations and ecosystems, we conclude that a major obstacle for synthesis across diverse host-pathogen systems is that data are collected on widely diverging scales with different degrees of resolution. This disparity not only hampers effective infrastructural organization of the data but also data granularity and accessibility. Comprehensive metadata deposited in association with genomic data in easily accessible databases will allow greater inference across systems in the future, especially when combined with open data standards and practices. The standardization and comparability of such data will facilitate early detection of emerging infectious diseases as well as studies of the impact of anthropogenic stressors, such as climate change, on disease dynamics in humans and wildlife.

1 **Genomics of hosts-pathogen interactions: challenges and** 2 **opportunities across ecological and spatiotemporal scales**

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42 **Abstract**

43 Evolutionary genomics has recently entered a new era in the study of host-pathogen
44 interactions. A variety of novel genomic techniques has transformed to the identification,
45 detection, and classification of both hosts and pathogens, allowing a greater resolution that
46 helps decipher their underlying dynamics and provides novel insights into their environmental
47 context. Nevertheless, many challenges to a general understanding of host-pathogen
48 interactions remain, in particular in the synthesis and integration of concepts and findings across
49 a variety of systems and different spatiotemporal and ecological scales. In this perspective, we
50 aim to highlight some of the commonalities and complexities across diverse studies of host-
51 pathogen interactions, with a focus on ecological, spatiotemporal variation, and the choice of
52 genomic methods used. We performed a quantitative review of recent literature to investigate
53 links, patterns and potential tradeoffs between the complexity of genomic, ecological and
54 spatiotemporal scales undertaken in individual host-pathogen studies. We found that the
55 majority of studies used whole-genome resolution to address their research objectives across a
56 broad range of ecological scales, especially when focusing on the pathogen side of the
57 interaction. Nevertheless, genomic studies conducted in a complex spatiotemporal context are
58 currently rare in the literature. Because processes of host-pathogen interactions can be
59 understood at multiple scales, from molecular-, cellular-, and physiological-scales to the levels
60 of populations and ecosystems, we conclude that a major obstacle for synthesis across diverse
61 host-pathogen systems is that data are collected on widely diverging scales with different
62 degrees of resolution. This disparity not only hampers effective infrastructural organization of
63 the data but also data granularity and accessibility. Comprehensive metadata deposited in
64 association with genomic data in easily accessible databases will allow greater inference across
65 systems in the future, especially when combined with open data standards and practices. The
66 standardization and comparability of such data will facilitate early detection of emerging
67 infectious diseases as well as studies of the impact of anthropogenic stressors, such as climate
68 change, on disease dynamics in humans and wildlife.

69

70 **Subjects:** Biodiversity, Ecology, Evolutionary Studies, Genomics, Zoology

71

72 **Keywords:** *Plasmodium*, MHC, immunotoxins, mucus, natural selection, GWAS, infectious
73 diseases, anthropogenic stressors, co-evolution, epidemiological surveillance

74 Introduction

75 Pathogens are widely agreed to be among the strongest agents of natural selection in nature,
76 and their influence on the genomes of host species is often readily detectable (Kosiol et al.,
77 2008; Enard et al., 2016; Quach et al., 2016; Shultz & Sackton, 2019). With the advent of rapid
78 DNA sequencing technologies, genetic studies of host-pathogen interactions have moved from
79 single gene perspectives to genome-wide approaches interrogating whole genomes of hosts
80 and/or pathogens. At the same time, these studies have begun to tackle an increasingly diverse
81 array of systems in both the field and laboratory, and have expanded from analysis of single
82 pathogens to multiple pathogens under a variety of conditions. Environmental factors and gene-
83 by-environment interactions, such as those beginning to be studied in microbiome research
84 (Libertucci & Young, 2019), are increasingly appreciated as important in modulating the severity
85 and fitness consequences of infections (Sekirov et al., 2010; Kamada et al., 2013; Villarino et
86 al., 2016; Chomwong et al., 2018). As genomic approaches become increasingly accessible
87 and affordable, it is becoming clear that the limiting factor in host-pathogen research is often not
88 the technical aspects of sequencing pathogens or host genomes, but rather the ecological,
89 immunological and epigenetic context in which genomic data are embedded (Kratochwil &
90 Mayer, 2015). To mention one example, post-translational modifications of proteins in the
91 mucus are known to play critical roles in pathogen defense in addition to genetic factors (Linden
92 et al., 2008).

93

94 Host-pathogen studies encompass an extraordinary variety of temporal and spatial
95 scales, including wide ranges of ecological settings and pathogen complexities - such as
96 experimental versus field studies or single versus multiple pathogens - as well as genomic
97 complexities, ranging from candidate gene studies to whole genome scans (Fig. 1A). Any single
98 study can be classified according to these scales, with concomitant benefits and deficiencies in
99 capturing the details of host-pathogen interactions in the real world. For example, studies aiming
100 to link the evolution of host and pathogen genomes and to detect genomic signatures of host-
101 pathogen interactions have arisen from searches for associations with single host candidate
102 genes, such as genes of the major histocompatibility complex (MHC) (Hill et al., 1991; Kaslow et
103 al., 1996; Wegner, Reusch & Kalbe, 2003; Meyer-Lucht & Sommer, 2005; Savage & Zamudio,
104 2011), to genome-wide scans for associations with resistance or susceptibility (e.g., (Fumagalli
105 et al., 2011; Bartha et al., 2013). We now have genomic insights into host-pathogen interactions
106 that stem from field studies investigating temporal and spatial patterns (Hill et al., 1991; Savage
107 & Zamudio, 2011; Penczykowski, Laine & Koskella, 2015; Bourgeois et al., 2017); to

108 experimentally evolving populations or ancient DNA studies encompassing hundreds or
109 thousands of host generations (Bos et al., 2011; Cairns et al., 2017; Tso et al., 2018; Spyrou et
110 al., 2019); to phylogenetic and comparative studies spanning tens of millions of years (Enard et
111 al., 2016; Koonin, Makarova & Wolf, 2017; Shultz & Sackton, 2019). This variety makes it
112 challenging to draw broad generalizations linking processes on different scales and to date, few
113 syntheses have attempted to bridge the many temporal and spatial scales on which host-
114 pathogen studies take place.

115

116 In this perspective, we aim to address the complexities and commonalities of diverse
117 studies of host-pathogen interactions through the lens of evolutionary genomics. We emphasize
118 the wide range of approaches used recently and focus primarily on evolutionary responses of
119 hosts to pathogens (Fig. 1). We first document the diversity of recent studies of host-pathogen
120 interactions through a comprehensive analysis of the recent literature on the subject. This
121 survey documents the sheer diversity of temporal and spatial scales on which host-pathogen
122 studies are conducted, but also reveals that the heterogeneity of results across studies, from
123 laboratory to field to experimental settings, poses a challenge for synthesis. Our survey
124 identifies gaps in emphasis on research on host-pathogen interactions, but also reveals
125 opportunities for discovering common principles and methodologies that are likely to drive the
126 research field forward. We then review major themes in the study of interactions between hosts
127 and pathogens in the wild. While daunting in terms of confounding variables, such studies
128 provide opportunities for studying the synergistic effects of anthropogenic change and the
129 evolutionary response to epizootics. At the same time, an increasing number of experimental
130 studies that examine the effects of multiple interacting pathogens on their hosts, or of host
131 microbiome on infection outcome, capture some of the reality of epizootics in nature. We
132 conclude that the full promise of genomic and other -omics approaches to further our
133 understanding of host-pathogen interactions will not be realized until these data are thoroughly
134 and consistently embedded in high quality, consistent, and reproducible ecological and
135 environmental metadata. Increased resolution of ecological metadata, smart databases that
136 facilitate collaboration and comparisons across studies, and deposition of voucher specimens
137 associated with specific studies in museum collections are just some of the ways in which
138 genomic data can realize their full potential. These new tools will facilitate the application of
139 knowledge of basic principles of host-pathogen interactions to real world problems affecting
140 wildlife, endangered species, and ultimately human welfare.

141

142 **Survey methodology**

143 This perspective piece is the result of discussions held during the “Origins of Biodiversity
144 Workshop” organized during May 29 – June 2, 2017 by Chalmers University of Technology and
145 the University of Gothenburg, Sweden, under the auspices of the Gothenburg Centre for
146 Advanced Studies (GoCAS). We gathered international scholars and students with
147 interdisciplinary backgrounds to discuss future perspectives of the study of host-pathogen co-
148 evolution in the genomic era. During the workshop we identified major directions that have been
149 enabled by advances in genomic techniques and in particular we highlight the resulting diversity
150 of host-pathogen studies in their ecological, temporal and genomic detail at which they are
151 studied. Our goal is not to provide a complete overview of the host-pathogen literature, but
152 rather illustrate the diversity of research undertaken in the field and the associated challenges
153 towards a comparable and inter-communicative understanding of causes and consequences of
154 host-pathogen interactions across systems. To quantify currently studied dimensions (ecological
155 complexity, spatiotemporal scope and genomic scale; Fig. 1A) of host-pathogen research we
156 conducted a literature search on Web of Science (accessed August 30, 2018) with the following
157 search query: (host-parasite* OR host-pathogen*) AND (genomic*). We refined the search hits
158 by document type to include only articles, covering the publication years 2014-2018, and
159 excluding studies with no genomic aspect. We scored 263 papers based on broadly defined
160 categories for each scale defined in Table 1. The reference list and scoring results can be found
161 in Supplementary Table 1. We used Spearman’s rho to assess the rank based association
162 between scales and adjusted p-values for multiple testing (Benjamini & Hochberg, 1995).
163

164 **Understanding the diversity of host-pathogen studies across** 165 **genomic, ecological and spatiotemporal scales**

166 We have outlined in the introduction that the published literature on host-pathogen interaction
167 spans a diverse range of genomic, ecological and spatiotemporal scales. However, how the
168 current published literature is distributed within this multidimensional space has not been
169 mapped out (Fig. 1A). We thus first performed a literature search to classify and quantify the
170 distribution of studies across these three scales. To understand the range of investigation at the
171 genomic, ecological and spatiotemporal scale in recent studies on host-pathogen systems, and
172 to discern where gaps in recent efforts might persist, we reviewed 263 studies of host-pathogen
173 interactions published in the period between 2014-2018 (see Table 1 for scoring categories and
174 Survey Methodology for specific details). A better understanding of the current placement of

175 host-pathogen studies should help us gain a better insight on how genomics can contribute to
176 the understanding of host-pathogen interactions that are being studied from the perspectives of
177 hosts or pathogens but also at such various level of biological detail.

178

179 We scored each study on three scales: genomic complexity, temporal and spatial
180 complexity, and ecological complexity (Table 1). We found that high genomic resolution (mean
181 score = 5.4 ± 2.1 SD, range = 1-7) at the level of the whole genome is employed to investigate
182 questions that span the whole range of ecological scales, from theoretical to multi-species
183 natural systems with environmental variability (mean score = 4.1 ± 2.4 SD, range = 1-9; Fig.
184 1B). Investigations of pathogen genomics dominate the dataset, whereas genomic
185 investigations of hosts are less common and more often examine a reduced representation of
186 the genome, transcriptome, or proteome. Studies encompassing the interaction of both host and
187 pathogens simultaneously are rare. Genomic techniques are rarely used to address complex
188 spatiotemporal scales (mean score = 4.1 ± 2.3 SD, range = 2-11), such as throughout the
189 geographical range of a species, or across multiple different species. (Fig. 1C). Intriguingly, with
190 increasing ecological complexity in a study, more complex spatiotemporal scales are also
191 addressed (Fig. 1D). However, only a few studies are classified as complex in terms of
192 spatiotemporal setting: Across all studies, spatial (mean score = 1.6 ± 1.0 SD, range = 1-5) and
193 temporal scores (mean score = 2.4 ± 1.7 SD, range = 1-6) are on average low. In particular,
194 studies of complex spatial scales, such as interrogation across multiple populations across a
195 species' range, are virtually missing.

196

197 The evaluation of published studies on host-pathogen systems not only reveals the
198 expected recent increase in whole genome datasets for a broad range of host-pathogen studies,
199 but also the gaps in addressing complex systems on ecological and spatiotemporal scales.
200 Perhaps most critically, we suspect that the quantity and complexity of the sequence data in
201 many recent and ongoing investigations of host-pathogen interactions pose increasing
202 challenges for comparisons across studies. The lack of comprehensive cross-taxon comparative
203 databases of host-pathogen interactions likely impedes the synthesis of individual host-
204 pathogen studies and translation of new knowledge into solutions for real world problems. In the
205 real-world, (a) pathogens attack hosts in the context of changing host environments, (b) these
206 environments are increasingly impacted by anthropogenic forces such as climate change, and
207 (c) are usually characterized by diverse communities of pathogenic and non-pathogenic

208 organism. Our cross-section of recent studies of host-pathogen interactions suggests that these
209 complexities are rarely captured in a single study.

210

211 **Disentangling hidden histories in genes and genomes of hosts and** 212 **pathogens**

213 The pathogenic lifestyle is ubiquitous across the tree of life, and pathogens are estimated to
214 represent a substantial proportion of the diversity and biomass of many ecosystems (Padra et
215 al., 2018). The advent of high throughput sequencing has facilitated the discovery of numerous
216 previously unknown pathogens and complex host-pathogen life histories, while metagenomic
217 approaches have allowed for the classification of host- and pathogen-associated microbial
218 communities, which have often been linked to successful pathogen infection and disease
219 development (Sekirov et al., 2010; Kamada et al., 2013). This large increase in the number of
220 observed pathogen strains and species along with associated microbes suggests that a
221 pathogen rarely occurs alone, and instead may commonly be a member of a larger community
222 (Robinson, Bohannan & Young, 2010; Schmid-Hempel, 2011; Gregory et al., 2019). Hence,
223 understanding the interplay between multiple pathogens and associated microbiomes requires
224 disentangling several levels of complexity. It is also crucial to gain an understanding of the
225 fitness effects of each putative pathogen on its host, because the magnitude of the fitness cost
226 (i.e. virulence) of a pathogen during infection determines its place on the mutualist-pathogen
227 continuum. In principal, this requires demonstration of a fitness cost to the host, yet
228 demonstrating fitness effects of many putative pathogens in nature is challenging and often
229 requires datasets that are much larger than those obtained in a typical field study [see Box 1].
230 Importantly, the fact that measuring the fitness consequences of infections in wild animals is
231 challenging does not imply that pathogens are insignificant selective agents in the evolution of
232 host genomes. In fact, pathogens are widely regarded to be among the strongest selective
233 agents. Mutations conferring moderate or large benefits of resistance to hosts can become
234 readily fixed by selection and are detectable through genome scans (Nielsen, 2005; Vitti,
235 Grossman & Sabeti, 2013; Haasl & Payseur, 2016). Thus, comparative and population genetic
236 studies of host genomes present compelling approaches for studying the presumed impact of
237 pathogens (Fig. 2).

238

239 Genetic variation is typically studied at different levels, such as across species (Fig. 2A),
240 across populations (Fig. 2B), within populations (Fig. 2C) or through time (Fig. 2D) to

241 disentangle the underlying genetics of host-pathogen interactions. For this purpose, two main
242 approaches are typically employed. On the one hand, the underlying genetic architecture can be
243 inferred using genotype-phenotype association studies. The statistical association between
244 genomic loci and host-pathogen phenotypes is interpreted as evidence for the underlying
245 genetics of a given phenotype (Hirschhorn et al., 2002). On the other hand, instead of
246 determining fitness costs of pathogens in single experiments or surveys (see also Box 1),
247 biologists have turned to signals of natural selection over evolutionary time as recorded in host
248 genomes (Sabeti et al., 2006). While these genome scans typically cannot directly test the
249 causal selective agent, they do provide insight into the possible biological processes that are
250 adapting most rapidly in host genomes (Biswas & Akey, 2006). Indeed, analysis of signatures of
251 selection in host genomes identified pathogens as the most likely drivers of the observed
252 patterns in a number of studies. For example, in *Drosophila*, Sackton and co-authors (2007)
253 identified that a class of immune genes that directly interact with pathogens, such as receptor
254 genes, exhibited a high proportion of genes under positive selection compared to genome-wide
255 observations. Similarly, across mammals, viral interacting proteins have stronger signals of
256 adaptation than other protein-coding genes across the genome (Enard et al., 2016), and more
257 of these genes than expected by chance are also evolving by positive selection in birds (Shultz
258 & Sackton, 2019).

259

260 A combination of selection scans and association studies has revealed important
261 insights into differences in infectious disease susceptibilities, the identification of specific
262 protective genes and alleles, and their evolutionary origin in humans, the most intensely studied
263 organism with respect to disease (Nielsen et al., 2005; Kwiatkowski, 2005; Williams et al., 2005;
264 Karlsson, Kwiatkowski & Sabeti, 2014; Malaria Genomic Epidemiology Network et al., 2015;
265 Enard et al., 2016; Enard & Petrov, 2018). There have been similar advances in the
266 understanding of the underlying genetics of natural host-pathogen systems in the wild. For
267 example, Bourgeois *et al.* (2017) was able to confirm and refine previously identified quantitative
268 trait loci that confer resistance in the planktonic crustacean *Daphnia magna* to the pathogen
269 *Pasteuria ramosa*. Furthermore, investigations of signals of selection have identified additional
270 genomic regions consistent with the evolution of resistance that were not identified by
271 association approaches. Such loci present further candidates moderating the host-pathogen
272 interactions, but without a clear association with specific phenotypic traits, evolution in response
273 to other environmental variables correlated with pathogens often cannot be excluded (Bourgeois
274 et al., 2017).

275

276 Despite the success of genome-wide associations and selection scans to find genomic
277 evidence of pathogen pressures on hosts, simultaneous genomic investigation of the co-
278 evolutionary dynamics between host and pathogen within a single system remain rare (see Fig.
279 1). Indeed, today, few systems have the genomic resources available to be truly interrogate
280 ongoing genomic changes between pathogen and host in parallel. One such example is Bartha
281 *et al.* 2013, who identified linked sequence variants between humans and HIV through genome-
282 wide-association scans. This study highlighted both host and viral loci that are potentially
283 involved in the co-evolutionary dynamics between host and pathogen. Additionally, emerging
284 studies of experimental evolution in the field or laboratory, or multigenerational sampling of
285 natural populations of hosts and pathogens, have successfully identified novel adaptive alleles
286 in both hosts (Scanlan *et al.*, 2015; Cairns *et al.*, 2017) and pathogens (Pal *et al.*, 2007). The
287 reciprocal nature of the interactions between host and pathogens over time naturally lead
288 researchers to ask whether host and pathogen species co-diversify over evolutionary time and
289 to what extent genomics can inform the underlying processes. Indeed, attempts to detect co-
290 speciation among hosts and pathogens date back to the beginning of the 20th century
291 (reviewed in (Vienne *et al.*, 2013). However, inferring co-evolutionary history through
292 comparisons of host and pathogen phylogenies is challenging. For example, such comparisons
293 can mistake a host shift followed by co-diversification as co-speciation (Vienne, Giraud &
294 Shykoff, 2007). The former mechanism is more consistent with empirical data that suggests that
295 the level of co-evolution necessary to drive co-speciation of host and pathogen is rarely
296 encountered in nature (Vienne *et al.*, 2013). As models of molecular adaptation and gene tree
297 evolution improve, we may be able to identify phylogenetic congruence at the gene-tree level or
298 signatures of selection that co-vary among hosts and parasites with more confidence. In turn,
299 we might be better able to interpret results in the light of co-diversification vs co-speciation of
300 studies, such as by Tso *et al.* (2018), where a pathogenic strain of *Candida albicans* evolved
301 into a gut symbiont in mice in just ten weeks. Parallel genomic analyses of the pathogen
302 showed that genes involved with an important virulence factor in *C. albicans*, the hyphal
303 morphogenesis program, had undergone rapid degeneration via both point mutations and
304 deletions.

305

306 **From candidate genes to whole genome analysis**

307 As outlined above, the co-evolution of host and pathogens can result in distinct and measurable
308 genomic signatures of selection, which can reveal the genetic mechanisms by which hosts and

309 their pathogens interact. The genomics revolution has spurred the transition from single-
310 candidate gene studies to genome wide analyses of hosts and pathogens. Historically, a
311 number of different candidate host immune genes families have attracted particular attention for
312 studies of host-pathogen interactions, including components of the innate immune system such
313 as toll-like receptors (TLRs) (Tschirren et al., 2013; Zhang et al., 2014; Zhang, Lun & Tsui,
314 2015; Shan et al., 2018), interferons and antimicrobial peptides (Clark & Wang, 1997;
315 Tennessen, 2005; Franzenburg et al., 2013; Carlin et al., 2018). These and other studies show,
316 both in vertebrates and insects, widespread signatures of positive selection and rapid evolution
317 in genes of the innate immune system (Świderská et al., 2018; Harpur et al., 2019; Adrian et al.,
318 2019). Gene expression studies have also revealed widespread activation of host innate
319 immune genes upon natural or experimental infection with pathogens, such as *Pseudomonas*
320 and *Daphnia* (Kumar et al., 2018). As such, these studies have contributed much to our general
321 understanding of the host's responses to pathogen exposure and common pathways to
322 resistance evolution over time.

323

324 The candidate gene family that has attracted the most attention in ecological and
325 evolutionary host-pathogen studies, at least in vertebrates, is the major histocompatibility
326 complex (MHC) (Hughes & Nei, 1988; Bernatchez & Landry, 2003; Meyer-Lucht & Sommer,
327 2005; Spurgin & Richardson, 2010). MHC genes encode cell-surface molecules that play a
328 central role in pathogen recognition as part of the adaptive immune response. T-cells act to
329 destroy infected cells both directly, as cytotoxic T-cells, and indirectly, as T-helper cells which
330 activate other immune cells, but they can only determine what is self or foreign from peptides
331 presented by MHC molecules. The number of MHC gene copies carried by individuals varies
332 widely between, and even within, species (Kelley, Walter & Trowsdale, 2004; Cheng et al.,
333 2012; Lighten et al., 2014). Additionally, the allelic diversity recorded within and between gene
334 copies makes the MHC genes the most polymorphic loci to date (Reche & Reinherz, 2003;
335 Robinson et al., 2015). This exceptional polymorphism is believed to be primarily maintained by
336 selection from a wide range of pathogens (Prugnolle et al., 2005; Qutob et al., 2011). Overall, it
337 is clear that MHC genes play a pivotal role in the fight against pathogens and numerous studies
338 have established associations between MHC genotypes and infections with a particular
339 pathogen (Kaslow et al., 1996; Meyer-Lucht & Sommer, 2005; Oliver, Telfer & Piertney, 2009;
340 Bolnick & Stutz, 2017).

341

342 Although the candidate gene approach has been the standard method for studying
343 immune genes in the context of host-pathogen interactions, the rapidly decreasing costs of high
344 throughput sequencing are making whole-genome approaches much more feasible. Whole-
345 genome population genetic and comparative genomic studies allow unbiased detection of
346 regions of the genome that are evolving non-neutrally across a variety of time scales. When
347 combined with functional annotations or association studies, such comprehensive genome-wide
348 surveys permit incisive tests of the effects of pathogens on host genomes that are simply not
349 possible from candidate gene studies. Furthermore, whole genome studies are not restricted by
350 *a priori* predictions of which genes are important in responding to pathogen challenges. Thus,
351 whole-genome approaches offer the potential to reveal new unbiased insights into the genetic
352 basis of host-pathogen interactions, e.g. (Enard et al., 2016; Shultz & Sackton, 2019). Since
353 multiple genes are most often involved in a host's response to a particular pathogen, whole-
354 genome approaches also have the potential to reveal these understudied polygenic responses
355 (Daub et al., 2013). However, a major caveat associated with the whole-genome approach is
356 that genomic regions of high repeat content, or highly duplicated genes, often do not assemble
357 well or at all, whether considering host or pathogen. Genome assembly problems may be a
358 significant disadvantage for host-pathogen studies given that some key genes which play a role
359 in innate and adaptive immunity are not only highly duplicated but also to some degree
360 physically linked in the genome, such as the beta-defensin and MHC genes (Kaufman et al.,
361 1999; Hellgren & Ekblom, 2010; Balakrishnan et al., 2010). Improved sequence and scaffolding
362 techniques are being developed to remedy problems of assembling such regions (Dilthey et al.,
363 2015) and some may be possible to overcome with long-read sequencing methods. Thus, while
364 the whole-genome approach may become a gold standard for many host-pathogen studies in
365 the future, we currently see a continued need for sequencing methods that target focal genes, in
366 particular in studies of non-model species.

367

368 **Genomic detection and surveys of pathogens**

369 Ever since the invention of the polymerase chain reaction, molecular approaches have
370 continuously provided sensitive methods for the detection of pathogens, often without prior
371 separation from the host tissues (e.g. malaria pathogens Snounou et al., 1993; Hellgren,
372 Waldenström & Bensch, 2004). High throughput sequencing techniques have now become
373 pivotal for both detection and identification of new pathogens, especially in cases of emerging
374 infectious diseases, and in pathogens with complex life histories and co-infections (Blasco-
375 Costa & Poulin, 2017). Furthermore, unmapped reads in host genome projects are likely a

376 fruitful source of undiscovered pathogens (Laine et al., 2019). Improved pathogen genomics
377 holds great potential to advance our current understanding of host-pathogen interactions in
378 several ways: From an epidemiological perspective, it allows one to reconstruct the spatial
379 spread of pathogen invasion, illuminates pathogen population dynamics, and enables
380 forecasting of future infection scenarios. Although this has been possible previously by using
381 only a few key genetic markers from samples that spanned decades in time, such as in studies
382 of influenza or rabies virus (Biek et al., 2007; Streicker et al., 2016), whole genome information
383 now allows for high-resolution characterization of outbreaks over shorter timescales (e.g. Ebola
384 (Dudas et al., 2017) and Zika (Faria et al., 2017; Grubaugh et al., 2017)). In addition, open
385 source genomic data-sharing sites and analysis platforms like nextstrain
386 (<http://www.nextstrain.org/>) are invaluable to explore pathogen time-space variation in real-time.
387 Moreover, genomic analyses of dated pathogen samples have proven successful in inferring
388 directionality of pathogen spread, for example, among wildlife and livestock, thus informing
389 effective control measures (Kamath et al., 2016).

390

391 However, many technical challenges still remain for such approaches, especially in
392 situations where pathogens cannot be physically separated from hosts (see Box 1 for an
393 example). For example, pathogen DNA typically makes up only a small fraction of the total
394 extracted DNA from samples of infected hosts, and host samples must therefore be sequenced
395 at >1000X to obtain a 10X coverage of the pathogen (Videvall, 2019). A large number of
396 enrichment protocols for high-throughput sequencing methods have been developed to facilitate
397 the detection and quantification of pathogens. These enrichment protocols are often efficient
398 ways of increasing the ratio of pathogen to host DNA. Before DNA extraction, intracellular
399 pathogens can sometimes be isolated from infected host cells using cell-sorting or laser-capture
400 microscopy techniques (Saliba et al., 2014; Wang et al., 2015), or separated from the host cells
401 by targeting different life stages (e.g. gametes, spores) (Palinauskas et al., 2013). After DNA
402 extraction, selective whole-genome amplification can specifically enrich for pathogen sequences
403 in various ways: (i) by using oligos that are more abundant in the pathogen genome (Melnikov
404 et al., 2011); (ii) by targeting differences in methylation between host and pathogen genomes
405 (Gómez-Díaz et al., 2012); or (iii) by sequence capture enrichment protocols for pathogen DNA
406 (Tagle et al., 1993). When enrichment protocols are not feasible, host and pathogen associated
407 reads can often be separated *in silico* using reference sequence databases. In such cases, low-
408 coverage detection of genome fragments of pathogens in host genome sequencing reads is a
409 straightforward and fruitful approach (e.g., Laine et al. 2019). Using this approach, putative RNA

410 viruses of *Drosophila melanogaster* were identified from de novo assembled RNAseq reads
411 (Webster et al., 2015). Dual sequencing analysis of both host and pathogen can be further
412 exploited to characterize the physiological response throughout the course of an infection
413 (Florens et al., 2002; Jean Beltran et al., 2017). However, sequencing coverage and cost are
414 major factors determining feasibility and scope of a study. Enrichment and optimization of
415 protocols carry the caveat that they are study specific and, in many cases, not universally
416 applicable.

417

418 Simultaneous genome sequencing of multiple species – metagenomics - can help the
419 field expand beyond the two-organism framework (Westermann, Barquist & Vogel, 2017), as
420 has been most extensively demonstrated in microbiome research in the context of host health
421 (Sekirov et al., 2010; Kamada et al., 2013). It is now clear that the whole microbial community
422 shape host health, but are also in turn selected for and manipulated by hosts (Näpflin & Schmid-
423 Hempel, 2016; Schwarz, Moran & Evans, 2016; Rolhion & Chassaing, 2016; Näpflin & Schmid-
424 Hempel, 2017). In particular, metagenomics is increasingly able to shed light on the function of
425 individual members of the microbiome, for example, by investigating metabolic pathways
426 present in the community (Lee & Hase, 2014). Similarly, sophisticated pathogen-specific
427 screening tools such as sequence chips with known pathogen probes can effectively screen
428 complex ecosystems for pathogens within the community and may identify potential disease
429 reservoirs (Bird & Mazet, 2018). Such approaches are employed by the PREDICT project of
430 USAID which attempts to identify new zoonotic threats in “hot spot” regions in Africa, Asia, and
431 Latin America by sampling wildlife (particularly non-human primates, bats, and rodents) as well
432 as people with close contact with wildlife (<http://www.vetmed.ucdavis.edu/ohi/predict/>).

433

434 Overall, genome-wide techniques and approaches provide us with an unprecedented
435 wealth of information upon which specific hypotheses can be formulated and experimentally
436 tested. A lingering limitation to the impact of such studies is low quality and poor annotation of
437 reference genomes, especially for non-model host species. This challenge considerably slows
438 our rate of discovery because many important parts of the host genome that respond to
439 pathogen infection may remain undiscovered if they do not assemble properly or lack known
440 gene annotation. Furthermore, relevant links to host-pathogen interaction could be missed
441 because the link between genetics and the expressed phenotype is only poorly understood (e.g.
442 the layer of mucus covering the mucosal surface in vertebrates whose composition is relevant
443 for the specific host-pathogen interaction; see Box 2).

444 **Infrastructural challenges of generalizing results across species and**
445 **systems**

446 The genomic data revolution driven by high-throughput sequencing has created numerous
447 exciting opportunities to study host-pathogen interactions in a multitude of systems in
448 unprecedented detail. This revolution extends to non-model organisms, although inference here
449 may be hampered by a lack of suitable and/or sufficient host or pathogen samples. Moreover,
450 even when such obstacles can be overcome, two major problems of relevance to this review
451 continue to constrain the full impact, reuse, synthesis and reproducibility of host-pathogen
452 studies, particularly for non-model systems: both involve the deposition and analysis of
453 associated sequence data. First, while it is standard practice for sequence data to be deposited
454 in well-curated, detail-rich national databases such as the National Center for Biotechnology
455 Information (NCBI), the European Nucleotide Archive (ENA), or the DNA Data Bank of Japan
456 (DDBJ), the associated biological metadata of these samples are often inadequate or
457 misleading due to various inconsistencies in available sample information that is being
458 deposited. This problem is not trivial to resolve in the context of host-pathogen studies, in part
459 because the complexity of the standardized metadata forms of these platforms for deposition
460 and retrieval of data (Dugan et al., 2014; Chang et al., 2016; Singh et al., 2019). Second, the
461 analysis of genomic data is preceded by a large number of computationally complicated pre-
462 processing steps. The choice of algorithm and parameters in this pre-processing procedure can
463 often have significant impacts on the final results but are generally inadequately documented
464 and communicated. Together, the missing metadata and the lack of transparency regarding
465 computational tools confound or even prevent robust meta-analysis and comparative studies;
466 and without meta-analyses and comparative studies, results from individual studies of various
467 host-pathogen systems cannot be integrated into a larger context.

468

469 Improving the availability of metadata and the transparency of computational tools
470 requires the compliance and openness of researchers to collect and analyze their data in a
471 standardized format, with the goal of making the data useable for comparative studies and
472 ultimately to make the data publicly available. Because the interactions between hosts and their
473 pathogens are inherently dynamic across space and time, accurate information on sampling
474 location and timing is essential information to include in metadata. This includes the host source
475 of isolation for pathogens and the infection prevalence of hosts. Importantly, the nomenclature
476 of genetically identical strains or species must be consistent. For example, despite being

477 genetically identical the haemosporidian lineage “*Haemoproteus* lineage 22” from birds, first
478 described in 2002, has repeatedly been named differently in publications appearing between
479 2002-2009: “AP21”, “COLL2”, “SWTH.H2”, and “WHA24” (Bensch, Hellgren & Pérez-Tris,
480 2009). An obvious first step is to improve the design, user-friendliness and programming
481 interface of currently existing popular databases for metadata. An integration of a large amount
482 of data sources has been developed for some systems, such as haemosporidian pathogens in
483 birds (MalAvi database, (Bensch, Hellgren & Pérez-Tris, 2009), or influenza viruses (GISAID
484 database, Yuelong Shu, 2017). Such efforts should ideally be extended to all host-pathogen
485 systems and are being realized more systematically under the umbrella of The Eukaryotic
486 Pathogen Genomics Resource (EuPathDB), a database of pathogen genomic data that
487 currently includes a dozen pathogen groups (Aurrecochea et al., 2017).

488

489 Similar to metadata documentation, detailed documentation of analysis tools and
490 parameter choice is becoming more widely advocated (Nature Editorial, 2017). The use of
491 scripted pipelines and version-controlled analyses has been advocated to address these
492 challenges (e.g. Nunez-Iglesias, 2015). At the most basic level this includes a scripted analysis
493 that does not require manual command input and thus is completely repeatable given the same
494 raw data (and sufficient computational time) (Beaulieu-Jones & Greene, 2016). Hence,
495 depositing analysis scripts in repositories such as Dryad or Github will become an important
496 component of comparative biology in the genomic era in general and in particular of host-
497 pathogens interaction studies. At its best, automated and curated pipelines that allow
498 continuous reanalysis of new and existing data will become an ambitious future goal for
499 comparative studies (e.g. The Lair (pachterlab.github.io/lair/about/)). Although such practices
500 will improve reproducibility of studies and integration of results across studies, such
501 improvement does not necessarily ensure and validate choice of appropriate methods
502 (Lotterhos, Moore & Stapleton, 2018). Nevertheless, extending automated analysis to
503 organisms with more limited genomic resources, which might permit linking of metadata (such
504 as whether a study is experimental or naturally observed) with sequence data across studies
505 would open up exciting frontiers in comparative studies of host-pathogen interactions across
506 different systems and beyond.

507

508 **Studying host-pathogen interactions in the Anthropocene**

509 The number of pathogen infections are predicted to continue to increase in the near future, as
510 climate change, human population growth and transportation impact the geographic distribution

511 and contact rate of hosts and pathogens (Altizer, Bartel & Han, 2011; Maganga et al., 2014;
512 Snäll et al., 2015). It has been estimated that wildlife is the source for 72% of emerging
513 infectious diseases in humans (Cleaveland, Laurenson & Taylor, 2001; Jones et al., 2008; Olival
514 et al., 2017) with recent examples including SARS, a virus in bats and small mammals; the
515 avian influenza type H5N1; and Ebola, originally a virus in fruit bats, which recently caused a
516 human catastrophe in western Africa (Dudas et al., 2017). Obviously, such pathogens can have
517 wide-ranging consequences on global societal stability and economy, and can have devastating
518 effects on natural populations (Daszak, Cunningham & Hyatt, 2000; Sachs & Malaney, 2002;
519 Bonds et al., 2009). In this context, rapid DNA sequencing technologies offer great promise for
520 our understanding of host-pathogen dynamics, and hence the ability to predict and control
521 disease epidemics (Wohl, Schaffner & Sabeti, 2016; Takahashi et al., 2018).

522

523 Natural systems are increasingly subjected to anthropogenic stressors, including climate
524 change, urban development, overexploitation, pollution, noise, and transport (Gerber et al.,
525 2014). In recognizing that no host-pathogen system exists in isolation, it is essential to
526 understand how such stressors affect the host's fitness, immune system and pathogen
527 susceptibility. For instance, immunotoxic contaminants can have substantial population level
528 effects by contributing to anthropogenic stress and infectious disease outbreaks (Desforages et
529 al., 2016). This is particularly true for marine and terrestrial top-predators, which, due to their
530 life-history and placement at the top of the food chain, accumulate high levels of ecotoxins.
531 Indeed, high tissue concentrations of persistent pollutants in Baltic seals in the 1970-80s were
532 associated with oviduct occlusions and impaired immune system, leading to sterility and
533 repeated infections (Bergman & Olsson, 1986), and recent work suggest that the same may be
534 true for a wide range of European dolphins and killer whales (Jepson et al., 2016). Such
535 increased levels of ecotoxins may explain the increasing prevalence and severity of diseases in
536 marine wildlife. A detailed understanding of the role of these and other stressors in host-
537 pathogen systems will require multispecies and multi-methodological approaches integrating
538 information at all levels of the system, including trophic interactions, resource availability, life-
539 history and population dynamics, as well as gene expression and selection.

540

541 Human intervention also has the potential to alter pathogen communities directly, both
542 by eliminating and by introducing pathogens (Daszak, Cunningham & Hyatt, 2000). Pathogens
543 can play crucial roles as ecosystem engineers (Thomas et al., 1999; Wood & Johnson, 2015).
544 Often, we lack the knowledge to accurately predict how the elimination of one pathogen will

545 affect the host population, other pathogens within the same host population, and their effect on
546 the ecological community (Rogalski et al., 2017). For example, the introduction of invasive
547 species often inadvertently results in the introduction of novel pathogens against which native
548 hosts may possess little or no protection (van Riper et al., 1986). Here again, major future
549 challenges include sample availability, ecological monitoring, and the collection and deposition
550 of appropriate metadata. Additionally, cross-disciplinary scientific integration and communication
551 between scientists, managers and decision-makers are crucial in order to advance global
552 health.

553

554 **Conclusions and prospects**

555 Innovations in genomic techniques have the potential to bring a synthesis to the study of host-
556 pathogen interactions across systems and environmental conditions. We highlighted several
557 recent trends in this perspective for genomic studies of host-pathogen systems: (i) evolutionary
558 genomics approaches have allowed the field to move from a candidate gene approach to
559 investigations at the scale of whole genomes; (ii) the use of genomics for the detection and
560 surveillance of host-pathogen systems; (iii) the challenges of the integrating natural history and
561 ecological metadata and genomic data across systems and timescales due to infrastructural
562 challenges of database integration and transparency; and (iv) the impact of anthropogenic
563 stressors on host-pathogen systems that have consequences for global health. Additionally, our
564 survey of the recent literature of ecological genomics of host-pathogen interactions revealed
565 that studies with spatially and ecologically complex settings are rare, as are detailed studies of
566 host genomic responses to pathogens. Any single host-pathogen study is constrained by limited
567 resources or genomic tractability, the geographical and evolutionary time scales involved as well
568 as environmental complexities. Accordingly, transparent and open science will help to achieve a
569 comprehensive understanding of host-pathogen interactions in general. This will contribute to
570 the integration of findings across the different scales (Fig. 1). A large repertoire of comparable
571 and inter-communicative studies will facilitate a more generalizable understanding of the causes
572 and consequences of host-pathogen interactions and a clearer roadmap to combating the
573 continuous threat of pathogens in a changing world.

574

Boxes:**Box 1: Demonstrating pathogen-induced fitness costs in the wild**

Determining where an organism lies on the mutualist-pathogen continuum requires an assessment of the fitness costs (i.e. virulence) elicited by a putative pathogen when it has established itself within its host in its natural habitat. In such a scenario, the feasibility of estimating fitness costs strongly depends, on the one hand, on the magnitude of the fitness effect and, on the other hand, the sample size. For example, severe negative fitness effects in birds due to infections by the introduced malaria pathogen *Plasmodium relictum*, have been readily demonstrated in several species of Hawaiian honeycreepers (*Drepanididae*) (van Riper et al., 1986). However, when *P. relictum* infects host species with which it has presumably co-evolved, observed fitness costs are lower (Bensch et al., 2007). Thus, hypothetically, demonstrating a negative fitness effect (i.e., mortality) of 5% year-on-year in natural populations (assuming a pathogen prevalence of 20% and an annual background mortality of 50%) requires a sample size of more than 2000 host individuals and the ability to accurately measure individual survival. The situation becomes even more complex when hosts are repeatedly exposed to the same pathogen and mortality varies across exposures. For example, if mortality is highest upon primary infection, in year two individuals that were unexposed in year one will be at a higher risk of dying than individuals that have been previously exposed. Often only long-term studies, such as that conducted by Asghar et al., (2014) on the effects of *Plasmodium* on lifetime fitness and survival of Great Reed Warblers, provide the sensitivity required to detect fitness costs.

Given that the ecological role of an organism can be dynamic, the fitness consequences for a host of a particular pathogen are strongly dependent on the environmental and genetic context. The most obvious illustration of this is variation in virulence associated with host switches: *Mycoplasma* infection in house finches as compared to other song birds (Ley et al., 2016), Ebola virus in humans as compared to bats (Leroy et al., 2005), or the morbillivirus Phocine Distemper Virus (PDV) in harbor seals as compared to other Arctic pinniped species (Härkönen et al., 2006) are all cases where virulence dramatically increased after switching to a new host. Second, another level of complexity presents itself in the cases of complex pathogen life cycles, where pathogens may require multiple host species for different developmental stages in order to complete their life cycle (Parker et al., 2003; Blasco-Costa &

Poulin, 2017). In cases such as these, it is often difficult to differentiate between pathogen species and different pathogen life stages morphologically. Third, infections by a single pathogen might actually be rare in nature, instead co-infections by multiple pathogen strains or species are likely to be the norm (Petney & Andrews, 1998). In this context, exposure history and the timing of infection might play crucial roles in terms of host fitness and pathogen virulence (Telfer et al., 2010; Ben-Ami, Rigaud & Ebert, 2011). Fourth, pathogen prevalence may vary across space and time and, hence, these patterns need to be taken into consideration in comparisons across scales (Thompson, 2009). This can be on a small scale within a host (e.g. between tissues), or across geographical space (e.g. between populations/species). For example, comparison of host and viral population structure suggests that dispersing male bats spread the rabies virus between genetically isolated female populations (Streicker et al., 2016). Fifth, hosts and their pathogens rarely interact in isolation but rather as part of a larger ecosystem, which might modulate how a pathogen interacts with its host and vice versa (Graham, 2008).

Overall, the availability of large genomic datasets has been pivotal in untangling each of the five levels of complexity. Nevertheless, relying solely on genetic data can be misleading. While new techniques help to identify new pathogens, ecological patterns, and link the genetic structure of host and pathogen populations, the resulting data are ultimately correlational and cannot establish any causal relationships without an experimental approach. For example, sticklebacks (*Gasterosteus aculeatus*) caught in a lake harbored more macroparasites than those from a river (Wegner, Reusch & Kalbe, 2003). With only this observation, one might be tempted to conclude that the sticklebacks from lakes were more susceptible to parasitism than those from rivers. However, subsequent experiments revealed that sticklebacks from lakes were less susceptible to pathogens, but probably experienced higher pathogen exposure (Scharsack & Kalbe, 2014). This illustrates the need for experimental studies to confirm causal relationships implicated by field data. However, experiments are restricted in the complexity they can represent (Plowright et al., 2008). In conclusion, the interpretation of genetic data without a deep understanding of the host-pathogen ecology, and vice versa, can be misleading.

Box 2: Barriers to infections – an example of difficulties linking genotype and phenotype

Hosts are continuously exposed to potential pathogens, yet the establishment of an infection upon encounter is a relatively rare event. Most pathogenic infections are successfully prevented by “simple” barriers, the host’s first lines of defense (McGuckin et al., 2011; Hall, Bento & Ebert, 2017). One of the most underappreciated pre-infection barriers in (non-human) ecology is the continuously secreted layer of mucus covering the mucosal surface in vertebrates (Fig. 3A), and the glycocalyx that covers other epithelial cells and surrounds some single celled organisms (Quintana-Hayashi et al., 2018). As opposed to the skin, which is a dry, acidic, and of much smaller surface, the mucosal surface is orders of magnitude larger and presents a semipermeable, humid environment that many bacteria and pathogens could thrive in. However, the mucosal surfaces are protected by several layers of defense that a pathogen has to circumvent to either gain access to close interactions with host cells, or entry into host cells, or transferring across the host epithelium. The first barrier the pathogens encounter is the continuously secreted mucus layer covering the cells and the epithelial glycocalyx (Quintana-Hayashi et al., 2018), into which a range of antimicrobial molecules are secreted, the bulk of this layer consists of a massive amount of highly diverse glycans (Fig. 3B). Among these highly diverse glycoconjugates, there are those that act as protection against infection by binding and disseminating the pathogen, act as steric hindrance or releasable decoys, but also those that act as receptors for pathogens and confer intimate adherence (Linden et al., 2008; Lindén et al., 2009; Padra et al., 2018).

In fact, across mammalian and teleost species, most known interactions between viral or bacterial pathogens and its host occur via host glycan structures (Aspholm-Hurtig, 2004; Linden et al., 2008; Venkatakrishnan, Packer & Thaysen-Andersen, 2013; Padra et al., 2014; Skoog et al., 2017). Interactions between host glycans and pathogens are thus central for host-pathogen specificity and virulence. As such, one would expect that host glycans and pathogen adhesins are subjected to strong selective pressure (Lindén et al., 2008; Lindén et al., 2010; Vitiaseva et al., 2015; Venkatakrishnan et al., 2017; 2019). While certain individual interactions between host glycans and pathogen adhesins have been dissected in detail (Rydell et al., 2011; Bugaytsova et al., 2017) it remains difficult to actually identify different glycoconjugate compositions and their underlying genetic basis.

While enzymes involved in glycan biosynthesis are easily identified based on sequence identity (curated collection: www.cazy.org; (Lombard et al., 2013) and make up about 5% of the total genome (Rini, Varki & Esko, 2015) the resulting glycan structures are governed by stochastic events, substrate availability and state of differentiation and physiological environment. Thus, with the currently available knowledge it is not feasible to predict glycan repertoire and biosynthetic machinery based solely on genomic and/or transcriptomic sequence data of the host. In addition, we currently lack ability to screen large sample sets for glycan repertoire because mass spectrometric based glycomics discovery is at its best only semi-automatic. Additionally, on the pathogen side, most adhesins of pathogenic organisms have yet to be identified and characterized.

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581

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Table 1 (on next page)

Table 1: Definition of categories for each scale and assigned scores used for the evaluation of host-pathogen literature.

Table 1: Definition of categories for each scale and assigned scores used for the evaluation of host-pathogen literature.

Score [†]	Genomic scale	Ecological scale	Temporal scale*	Spatial scale*
1	gene/ sequence fragment	none/ theoretical	none	none
2	full gene/ regulator	single species, laboratory system, environ. constant	single generation	local (one population)
3	gene family/ microsatellite	single species, laboratory system, environ. variable	few generations	intermediate (couple of populations)
4	whole plastid genome	multiple species, laboratory system, environment constant	many generations	species range
5	reduced genome representation	multiple species, laboratory system, environ. variable	speciation time (small tree)	global
6	exome/ transcriptome/ proteome	single species, natural system, environ. constant	speciation time (large tree)	
7	whole genome	single species, natural system, environ. variable		
8		multiple species, natural system, environ. constant		
9		multiple species, natural system, environ. variable		

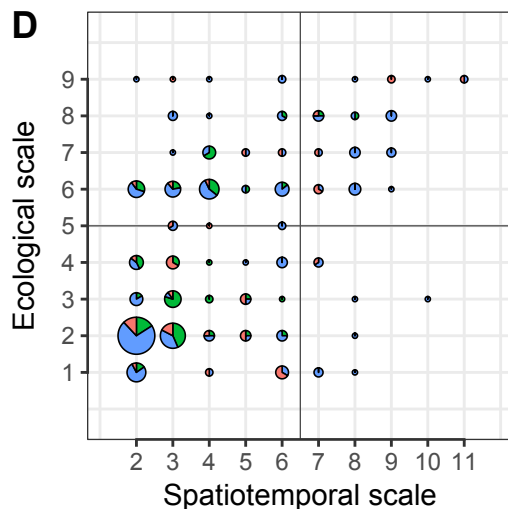
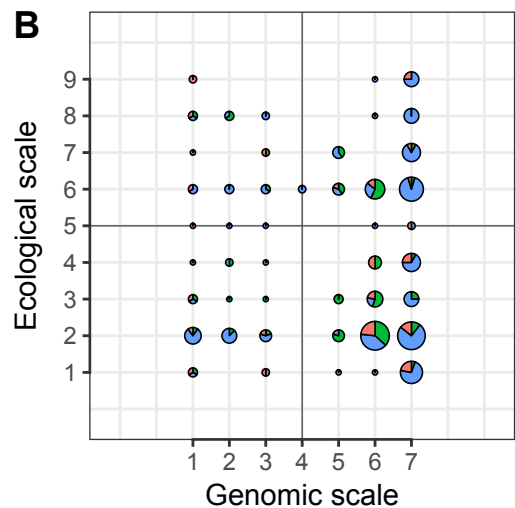
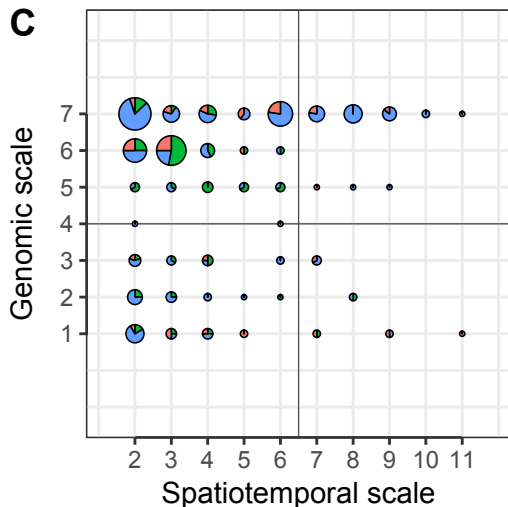
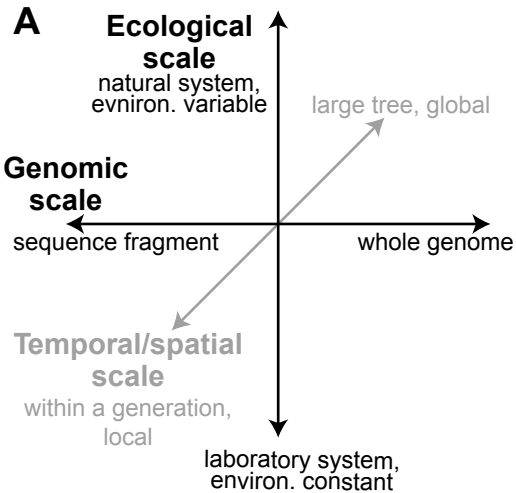
† see SI Table 1 for list of references and associated scoring results

* the spatiotemporal scale (Fig. 1) is the sum of the individual scores of the temporal and spatial scales

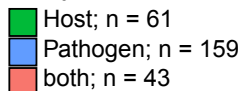
Figure 1 (on next page)

Figure 1: The diversity of recent studies of host-pathogen interactions.

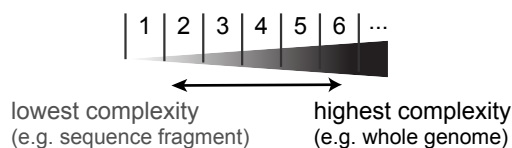
(A) Each of three scales of complexity – genomic, ecological and spatiotemporal – is represented as an axis in this illustration. A study of host-pathogen interaction is placed into this three-dimensional space based on the level of genetic, ecological, and spatiotemporal detail that is being studied (see Table 1 for scores of scales). **(B-D)** Pie charts summarize the results of the scores for the level of genetic, ecological, and spatiotemporal complexity investigated in host-pathogen studies published between 2014-2018. **(B)** The complexity of the ecological and genomic settings across studies are not correlated (Spearman's $\rho = 0.02$, p -value adjusted = 1.00; **(C)** nor are the genomic and spatiotemporal scale ($\rho = 0.16$, p -value adj. = 0.13). **(D)** In contrast, the ecological scale positively correlates with the score of spatiotemporal scale across studies ($\rho = 0.50$, p -value adj. = 0.00).



Study focus:



Scores of scales:



of studies:

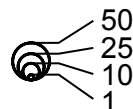
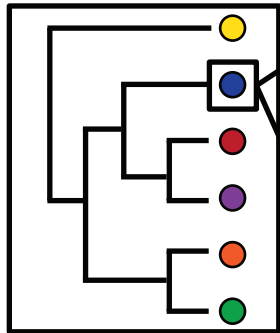


Figure 2(on next page)

Figure 2: Schematic illustration how genetic variation varies (A) across species, (B) across populations, (C) within a population, and (D) on an ecological time scale.

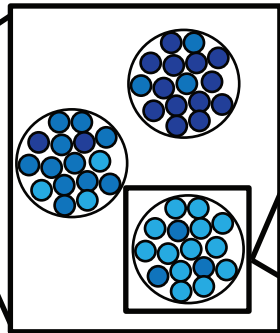
Comparative genomics across species can be used to identify genomic loci consistently under positive selection in particular lineages or all lineages **(A)**. Across populations **(B)**, population genomic variation in different geographic populations can be correlated with pathogen communities. Within a single population **(C)**, phenotypic variation among individuals can be linked to pathogen variation or differentially expressed genes with transcriptomics. Genome scans may also identify regions of the genome under selection. Finally, time series **(D)** either derived through experimental evolution or studies of ancient DNA or diachronic samples can be used to identify the dynamics of a phenotype or allele frequency through time.

A

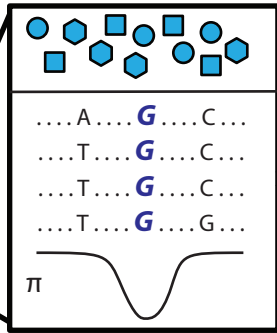


Comparative Genomics

B

Population Genomics
Community Variation

C

Transcriptomics
Infection Experiments
Selection Scans

D

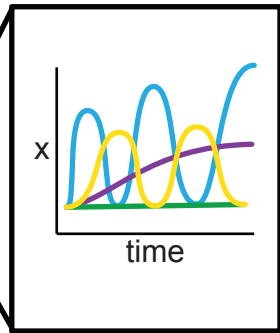
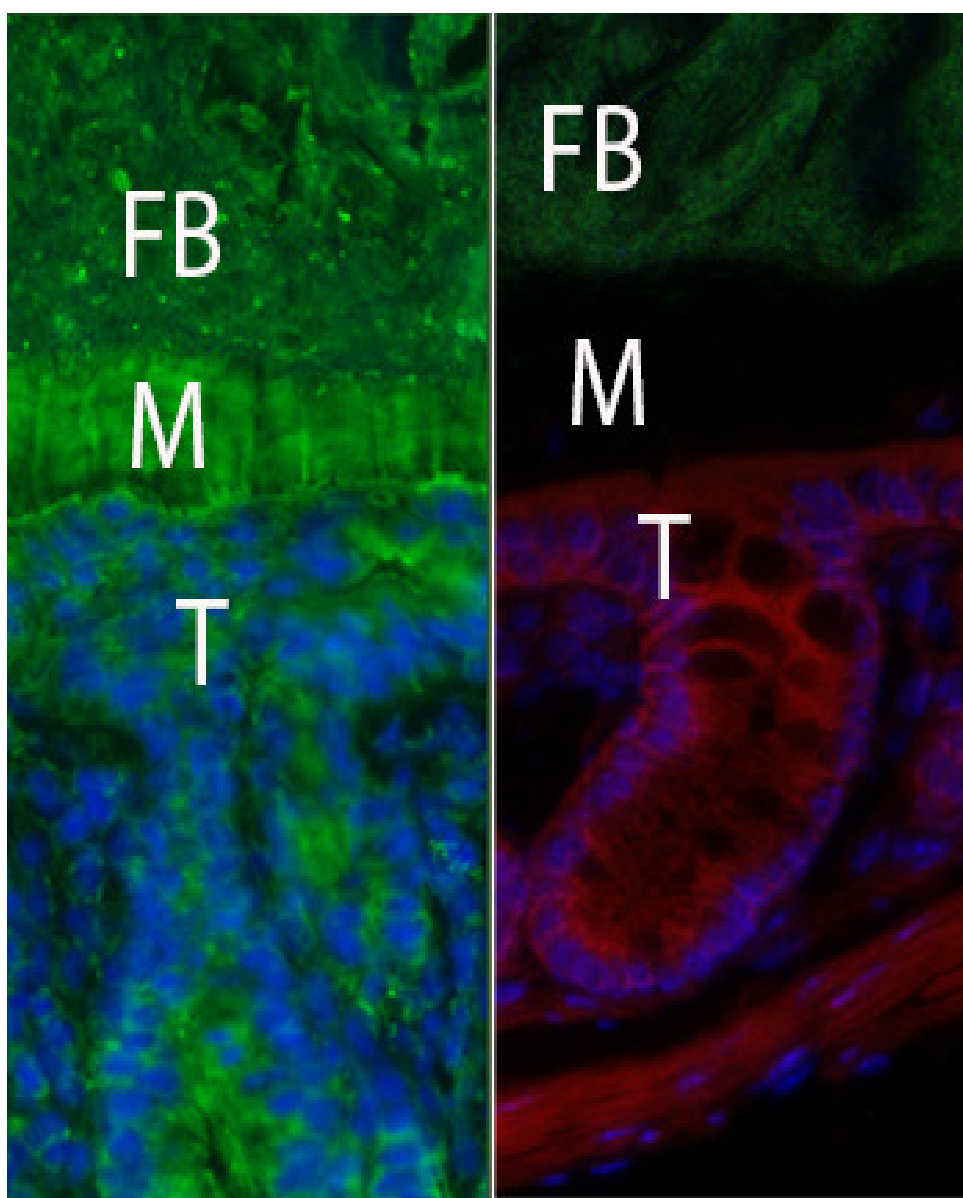
Time Series:
Experimental Evolution
Ancient DNA

Figure 3(on next page)

Figure 3: The mucosal layer.

(A) shows two staining variants of the colonic mucosal tissue (T) of a healthy mouse, where a mucus layer (M) keeps the majority of the fecal bacteria (FB) from direct contact with the surface of the epithelial cells. On the left side, the Muc2 mucin (the main component of the mucus layer) is stained in green and nuclei from the eukaryote cells in the tissue are stained blue. Muc2 is produced by cells in the mucosal tissue, secreted into the mucus layer, and present in degraded form in the fecal material. On the right side, the mucosal epithelial tissue is outlined with red, eukaryotic nuclei are purple, the mucus layer unstained (but clearly visible due to the absence of bacteria) and the bacteria are labelled green. Panel **(B)** gives an overview of glycan structures that build the mucus layer and glycocalyx. Glycolipids and glycoproteins are anchored in the eukaryotic cell membrane, and secreted mucins are highly glycosylated glycoproteins consisting of 70-90% of glycans that make up the bulk of the mucus layer. The glycans can be longer and more complex than depicted in this illustration. The glycans can be either *N*-linked (via Nitrogen in asparagine) or *O*-linked (via Oxygen in serine or threonine) to the protein core, and these two types of glycan chains differ with regards to biosynthetic pathway and structure.

A**B**