CHAPTER 10

HOW TO CATCH THE RED QUEEN?

DIETER EBERT

In 1980 Bill Hamilton published a paper with the attention-getting title 'Sex versus non-sex versus parasite',[1] in which he proposed that parasitic diseases are an important factor for the maintenance of sexual reproduction. This hypothesis came to be known as the Red Queen hypothesis because it is based on the idea that hosts and parasites are in a never-ending race, just as the Red Queen in Lewis Carroll's fairy tale cannot stop running, or else she would lose her position. Although, the Red Queen hypothesis attracted a lot of attention, more than ten years after it was proposed very little empirical work had yet been conducted on it. Surprisingly, the widespread support that the hypothesis had earned by the early 1990s among many evolutionary ecologists was not based on hard data, but on its plausibility and the increasing recognition that parasites are indeed everywhere. What was actually known about the interactions among hosts and parasites boiled down to only a few studies. Curt Lively, who worked on snails, and Paul Schmid-Hempel, who worked on bumble bees, were among the pioneers in the field.[2] However, one of the main reasons for the paucity of data was that there were not many host–parasite systems suitable for efficient experimental work to be conducted. In particular, systems with eukaryote hosts and rapidly evolving parasites had not been used much, although these were the systems where it was suspected that the hypothesis could most easily be tested.

Inspired by the elegance of the ideas involved in the evolution of host–parasite interactions, I decided to switch from life-history studies to evolutionary parasitology for my post-doc. As Bill Hamilton was the key proponent of the Red Queen hypothesis, I visited him in Oxford in the

Summer of 1990 to enquire about a post-doc in his group. Initially, Bill was rather sceptical, as he was not sure whether my background in *Daphnia* life history studies was suitable for a project on the Red Queen hypothesis. However, he liked *Daphnia* as an experimental system, and in the end we agreed that I would try to develop an experimental host–parasite system with *Daphnia*. I had had experience with *Daphnia* before, so we expected that this part of the work would not pose a problem.

AQ: please check the sentence "I had . . . problem".

When I joined Bill in Oxford for my post-doc, my first aim was to develop the *Daphnia* system and to use it for empirical work on the Red Queen hypothesis. Around Oxford, there were many suitable places to find *Daphnia*, and I soon had many cultures running, although the laboratory conditions where far from optimal for this type of aquatic work. A bigger problem was the parasites. As I had no experience with parasites, it took me much longer than expected to find them. From time to time Bill would have a look in the microscope to see what there was to find in *Daphnia*, but for several months we did not see anything resembling what we had hoped for. Finally, a visit to Jim Green's laboratory at St. Mary and Westfield College in London opened the door to the secret world of micro-organisms, showing that there was much to find. Nearly every individual *Daphnia*, in particular *D. magna*, was infected with one or another parasite, mainly bacteria and microsporidian parasites. Within a few weeks I was able to culture two species of parasites (the bacterium *Pasteuria ramosa* and the microsporidium *Glugoides intestinalis*, formerly called *Pleistophora intestinalis*), and I could concentrate on the second aim of my post-doc, namely to test the predictions of the Red Queen hypothesis experimentally.

During the course of my explorations in the world of parasites, another post-doc in Bill's group joined the project. Katrina Mangin shared much of my enthusiasm for the Red Queen hypothesis. On one occasion we discovered what we thought might be a new parasite species when we observed individual hosts carrying large amounts of small spore-like structures that we could not identify. After further investigations we found that only males seemed to be infected with this parasite, but that females could transmit this infection vertically to her offspring. Apparently the infection was virulent only in males, which made perfect sense to us, as a vertically transmitted parasite would curtail its own transmission if it harmed reproductive females. When we told Bill about this new parasite, he was eager to see it in the microscope. After he had a good look, he commented in a very polite way that he was not really sure whether this was a parasite, as he vaguely

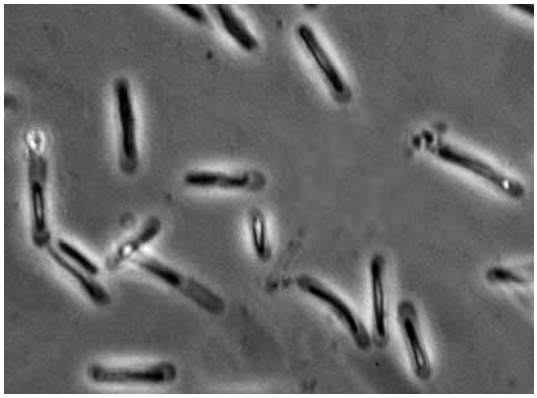

Figure 10.1. Sperm of *Daphnia magna*. (Photo and Copyright by Dieter Ebert).

remembered seeing sperm that looked like this (Fig. 10.1). We had not considered this possibility, although we also had never seen *Daphnia* sperm before: *Daphnia* research focuses largely on the females due to its asexual mode of reproduction. Well, after some further investigations we had to admit that Bill was indeed right. In our defence, we should add that *Daphnia* sperm looks very different from what sperm usually looks like. Only much later did I discover an old paper with drawings of *Daphnia* sperm that resembled our findings closely. Clearly Bill's wide experience with the biology and natural history of all kinds of unusual creatures had set him quickly on the right track.

After the system was up and running in the laboratory, Bill and I frequently discussed which predictions of the Red Queen hypothesis could be tested experimentally. This turned out to be more difficult than we both had anticipated. The published literature on this topic was of limited help. Mathematical models and computer simulations gave clear insights into certain aspects of host–parasite arms races, but it was often not clear to what extent the predictions of models could be used for real systems. For example, nobody knew the genetics that underlay host–parasite interactions. There were some data from plant–fungal system (the gene-for-gene model), but the proposed mechanism was not verified for animal systems. Most theoretical studies on the Red Queen hypothesis used the matching allele model, for which we have until now little empirical support. In contrast to these genetic systems, some verbal predictions were based on quantitative genetics models, i.e. offspring resemble their parents with regard to susceptibility and

resistance to infectious diseases. This idea, translated into a coevolutionary scenario, led to the somewhat obscure prediction that heritabilities of resistance should be negative. Other predictions of the Red Queen hypothesis being discussed at this time seemed to depend on contrasting points of view. For example, one could argue that sexual host populations should have less parasites than asexual populations, because genetic recombination helps them to fight parasites. However, one could equally well argue that sexual populations are sexual because they have more parasites and that the asexual populations can only be maintained because they have fewer or no parasites. It may be even more difficult: in dynamic interactions such as coevolving antagonists, the observed relationships between certain variables may change over time, and a clear picture may only become visible over a very long period of observation. The most often cited prediction of the Red Queen hypothesis is that gene frequencies should cycle over time. This prediction however, is very difficult to test, as it requires that we know the genes under parasite selection and that these genes can be traced in populations. Currently we do not know for any host–parasite system enough of the underlying genetics to trace cycling genes, not even talking about cycles in linkage disequilibrium among genes. Furthermore, we need to know on what time scales should we look for cycles. If cycles are slow (e.g. decades) we would hardly be able to see them within a normal research program. Years later, Dybdahl and Lively[3] circumvented the first problem by using clonal snails, in which the entire genome, including the resistance genes, form a linkage group. Surprisingly for many people, but good news for the Red Queen hypothesis, they found that even on short time scales (few years) clones frequencies can change drastically in response to parasites that attacked common clones over proportionately more often than rare clones. Unfortunately, there are only a few systems that allow such studies, excluding the observation of cycles for the majority of systems.

There was clearly some conceptual work to be done before I could start with the experiments. The paper to which this small and certainly rather personal article is the foreword, is the result of the discussions Bill Hamilton and I had over numerous teas drunk from styrofoam cups in the coffee area of the Oxford Department of Zoology. Bill's abstract sense for important biological relationships and my sense for what is a testable prediction did not always coincide. In numerous incidences, I dismissed Bill's suggestions as being untestable within the normal framework of our research possibilities.

Likewise, Bill dismissed many of my suggestions as already evident, not in need of further investigation. While his suggestions inspired me to think bigger, my suggestions may have helped him understand that what seemed clear to him was not necessarily acceptable for an experimentalist who views data points as conservative estimates of reality.

It quickly became clear that a key issue was the question of whether hosts or parasites are ahead in the arms race. At this time it was usually assumed that because parasites evolve quicker than their hosts, they are therefore ahead. If this were true, it would be rather simple to come up with clear-cut predictions regarding host–parasite arms races. However, nobody knew anything about the relative advantages of the two antagonists in the arms race. From bacteria-phage experiments we had learned that, rather than test patterns created by past evolution, we could allow evolution to happen under controlled conditions and then test the outcome. Applying this form of experimental evolution to evolutionary hypothesis was not yet widespread when we started our discussions and was until then a domain of evolutionary microbiologists. However, as Red Queen host–parasite arms races were assumed to happen with considerable speed, they seemed suited for experimental evolution. Thus, we decided to avoid the question of who is ahead in the arms race by making predictions for evolutionary experiments in which one antagonist would evolve, while the population of the other antagonist would be kept genetically constant. In this way, it would be clear that the evolving antagonist would eventually be ahead of its opponent. The predictions made for such experiments form the nucleus for the paper following this article, which we published about 2 years later.

A literature review of studies that allowed parasites to evolve on well-defined host lines provided some support for the predictions derived from the Red Queen hypothesis.[4] In this review, I examined the huge amount of medical, veterinary and agricultural literature on serial passage experiments. Typically, in these experiments, a novel, but related, host is artificially infected and the infection is then transmitted from one host individual to the next (e.g. by syringe transfer of blood). The host strains are usually well defined and of low genetic diversity (inbred lines, full sib families, clonally propagated cell lines). Despite the huge variation in the purpose and methodology of serial passage experiments, several studies consistently reported that parasite virulence increased during serial passage experiments as a result of within-host competition and that this increase in virulence depends on the host genotype. (Parasites passed through one host-type

become 'attenuated', i.e. their pathogenic effects are reduced in hosts different from those in which they were passed. Attenuated parasites are useful vaccines, for they can elicit an immune response without causing harmful effects, e.g. Sabin's polio vaccine, smallpox, rubella, measles, mumps.) Given these results, the question arises, 'Why does virulence not increase under normal, "non-passage" conditions?' The Red Queen hypothesis offers a solution (but not the only solution[4]) to this problem. During serial passage experiments, parasites are exposed to a narrow range of host genotypes. Infection of a novel host, usually a different host species or a different cell line, results in parasite attenuation, indicating that growth and virulence are adaptations to the host-genotype in which it evolved. Ebert and Hamilton[5] proposed that virulence does not usually escalate in natural populations because genetic diversity among hosts prevents the parasite from evolving host genotype specific virulence. In other words, because the parasite suffers from attenuation whenever host-to-host transmission occurs, it rarely has sufficient time to evolve high virulence on a single genotype. Genetic diversity among hosts hinders the escalation of virulence. Under such conditions, rare host genotypes have a selective advantage.

Serial passage experiments have not been designed to test the Red Queen hypothesis and therefore do not convince in all aspects. However, an increasing number of experimental studies have been published indicating that arms races, such as those envisioned by Bill Hamilton when he proposed the Red Queen hypothesis, are a natural part of host–parasite interactions. These arms races are not only intriguing by their complexity, but also by the strength and speed of their dynamics.

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SEX AGAINST VIRULENCE: THE COEVOLUTION OF PARASITIC DISEASES

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Abstract

Reciprocal selection is the underlying mechanism for host–parasite coevolutionary arms races. Its driving force is the reduction of host lifespan or fecundity that is caused by a parasite. Parasites evolve to optimize host exploitation, while hosts evolve to minimize the 'parasite-induced' loss of fitness (virulence). Research on the evolution of virulence has mostly emphasized the role of parasite evolution in determining virulence. However, host evolution, accelerated by sexual recombination, contributes to the evolution and expression of virulence as well. The Red Queen hypothesis predicts that genetic variation among host offspring facilitates selection for reduced virulence. Here, we outline a synthesis between current thinking about the evolution of virulence and the evolution of sex.

Parasites—here broadly defined as damage-producing organisms, including microbial pathogens, traditional parasites and small herbivores—are ubiquitous and influence either directly or indirectly almost every conceivable level of biological organization. The impact parasites have on the evolution and ecology of their hosts depends on their virulence, the driving force in host–parasite coevolution. Virulence, *per se* beneficial for neither parasite nor host, cannot be a property of a parasite alone; rather, it is a product of the host–parasite interaction. Different host genotypes from the same population do not suffer equally when infected with the same parasite strain, and different parasite strains cause variable levels of virulence in the same host genotype.[1–3]

Most studies on the evolution of virulence have concentrated on parasite evolution, assuming that virulence is maintained by genetic trade-offs between virulence and other fitness components of the parasite. For example, parasite-induced host mortality was shown to be negatively correlated with host recovery rate (which contributes to parasite mortality) in Australian rabbits infected with

the myxoma virus[4,5] and positively correlated with the multiplication rate of a microsporidian parasite in *Daphnia* hosts.[3] Therefore, it has been suggested that to maximize fitness a parasite should optimize the trade-off between virulence and other fitness components.[5] This optimality concept for the evolution of virulence, however, largely neglects genetic variation among hosts in their interaction with parasites. Such variation results in differential reproductive success among hosts and would, in the absence of parasite evolution, lead to reduced virulence. Given the high evolutionary rate of parasites,[4,6] host evolution can often be ignored in a first approximation, but for a better understanding of the evolution of virulence it is essential to understand the host's evolutionary response and in particular the role of genetic recombination in host evolution.

It has been suggested that sexual reproduction of hosts is a means to overcome the disadvantage of the low evolutionary rate that an asexual host would have in comparison with its rapidly evolving parasites.[7–9] Combining current theory of the advantage of genetic recombination and outbreeding with the theory on the evolution of virulence, one would predict that hosts continuously evolve to reduce virulence, while their parasites evolve to keep virulence as close as possible to an optimal level for their own life histories. In this arms race, a high evolutionary rate would benefit both opponents. Since parasites already have a very high evolutionary rate intrinsic to their short life cycle, hosts would be selected for increased evolutionary rates, even if this has costs. Sexual recombination could provide such an increase.[7,8,10,11] The principal underlying assumption for this hypothesis is that genetic variation for host–parasite interaction exists within populations and gives differential fitness to both the host and the parasite. Such genetic variation has been shown for different host–parasite systems [e.g. refs 2, 12, 13, including variation at the major histocompatibility complex (MHC) in relation to infectious diseases in humans (severe cerebral malaria[1] and chronic lyme arthritis[14])]. Given continuance of such variation, genetic recombination creates novel gene combinations. In a sexual population, every host constitutes a genetically unique environment for the parasite. Therefore, parasite adaptation to one host genotype is only of temporary benefit. Host diversity hinders evolution towards an optimal level of virulence, and we expect a level reflecting not only the evolution of the parasite to optimize host damage, but also the evolution of the host to minimize damage.[15,16]

Observing the Evolution of Virulence

The hypothesis that virulence in naturally coevolving populations is on average sub-optimal for the parasite allows us to make testable predictions.

If host evolution is experimentally restricted by reducing host genetic variability, parasites would be expected to adapt to the predominant host genotype by shifting virulence upwards, towards their optimum. There is some empirical evidence that virulence increases during adaptation to a new host genotype. Influenza virus increases in virulence and multiplication rate when propagated experimentally within chickens with the same genetic background.[17,18] Virulence of the measles virus increases when transmission occurs between siblings, compared with transmission between non-related members of the same host population.[19,20] The primate malaria agent *Plasmodium knowlesi* initially causes mild infections in humans; however, after 170 artificial human to human transfers, its virulence had risen sharply, indicating its adaptation to the new host environment (discussed in ref. 21). The well-known case of initial decrease of virulence of the myxoma virus in Australian rabbits does not contradict this. The above prediction assumes that host and parasites are in a dynamic balance and it is when this holds that slower evolutionary change of the host will allow the parasite to increase virulence. The myxoma virus, however, was released to control rabbits and was therefore chosen to be as deadly as possible.[4] Since it was in a non-natural host the optimal level of parasite virulence was far off the optimum—in this case, far above the optimum. The strong decline of myxoma virus virulence over the first few years after its release indicates the strong potential of parasites to respond to changes in their genetic environment. Although ecological factors might have contributed to all these described changes in virulence, adaptation to the novel host genotype appears to us to be the more likely explanation.

The adaptation of a parasite to a new host genotype that was formerly rare or absent often results in the loss or a decline of virulence in the host of origin.[17] This finding was used in the development of vaccines, by employing strains in immunization programs that became non-virulent for their normal host after being kept for some time in foreign hosts (e.g. vaccines against yellow fever virus and poliovirus.)[22,23] This process emphasizes the dynamic, 'Red Queen' nature of host–parasite interactions.[7,10]

Local Adaptation

Support for the coevolutionary hypothesis of virulence also comes from studies using the reverse of the above argument. Namely, a parasite that infects a novel host (a host of a different genotype or population from that to which the parasite is adapted) should initially have, on average, a lower virulence in this new host, compared with that in its original host. In nearly all experiments where parasites were brought into contact with novel hosts, virulence and transmissibility decreased; this has been shown for viruses,[24] fungi,[25]

helminths,[26,27] protozoans[3] and herbivores.[28–30] The reduction in virulence and transmissibility was stronger the more the novel hosts differed genetically from the host with which the parasite was associated before the experiment.[3] Experiments that did not find significant advantages for the parasite in its original host showed very high levels of genetic interactions between hosts and parasites[31–33] and this could contribute to the masking of local adaptation. Failure to detect local adaptation in cases where it is present can have various causes. Statistical power is weak when the within 'genetic unit' (e.g. host population, geographic area) variation is much larger than the variation explained by genetic isolation. Misjudgment of the scale of local adaptation might also lead to problems; for example, if parasites adapt to individual hosts rather than to host populations, detection of local adaptation across host populations might be difficult. Detection of local adaptation may also be hindered by acquired immunity of hosts, maternal effects on resistance, asymmetric gene flow between populations (source and sink populations) and insufficient time for adaptation. To our knowledge, no evidence has been presented against local adaptation.

Attention-attracting as they may be, cases where parasites showed devastating effects after accidental introduction into new host populations (e.g. rinderpest in Africa, Dutch elm disease, chestnut blight, HIV) appear to be exceptions.[3,13,16] There are likely to have been numerous failed introductions that have passed unnoticed. Most studies conducted under controlled conditions (see above) clearly show that parasites cause, on average, most harm in the host populations to which they are adapted.

Testing the Red Queen Hypothesis

In summary, genetic diversity of host populations appears to be crucial in hindering the parasite to evolve an optimal level of virulence. Sexual recombination benefits an outcrossing host through the production of variable offspring. In the context of host–parasite relationships, novel and rare genotypes have intrinsic advantages and may be selected. Asexual offspring, in contrast, cannot escape the antagonistic advances made during the previous generations by their parent's parasites. Genetic variability among hosts forces a parasite to adapt anew whenever it encounters a new host genotype.[10,34] The more different genotypes a host population consists of, the lower the frequency of each and the smaller the chance that a parasite will encounter the same genotype in successive hosts.

So far, direct evidence for the benefits of genetic diversity for host populations is weak, although numerous studies suggest such benefits. The vulnerability of monocultures to pathogen attack is notorious. Cereal

monocultures have been shown to be more prone to attack by rapidly evolving, clone-specific, fungal diseases than genetically mixed cultures are.[35] Also, virulence of viruses was suggested to be higher in human populations with low genetic diversity at their MHC than in MHC-diverse populations.[20] Studies in natural populations are difficult, because frequency dependence of the host–parasite arms race is time-lagged, and therefore one cannot expect a positive correlation between the parasite prevalence and frequency of host clones.[36]

The theory that sex is advantageous in the presence of rapidly evolving parasites[7,8] became known as the Red Queen hypothesis. Support for the 'sex against parasites' hypothesis has come mainly from comparative studies (e.g. Refs 9, 37–39). Experimental studies are so far limited, with some support coming from the effects of herbivores on long-lived plants.[40,41] There is a strong need for experimental tests to clarify our understanding of arms races and the advantage of outbreeding. Boxes 1–4 summarize testable predictions derived from this review. Our predictions are stated for clonal host populations, but they are valid for all host organisms that can be brought to a lower level of genetic diversity than that found in normal wild populations (for example, by cloning, by using offspring from crossed inbred lines, or by using full-sib families). It is also helpful if strains of parasites and/or host can be 'genetically frozen' over time, to be used as a control in later experiments.[12] We hope that these predictions will stimulate research on the coevolution of sex and virulence.

Box 1. Testing the Red Queen: do parasites express higher virulence in host genotypes to which they are adapted?

(1) Variation in virulence reactions is expected in both hosts and parasites. 'Wild-sampled' hosts will vary in fitness when exposed to a single parasite strain and 'wild-sampled' parasite strains will vary in virulence and reproductive success when applied to a single host line.
(2) Wild-sampled parasite strains will initially evolve an increase in virulence and reproductive success when kept in monoclonal host populations.
(3) Parasites taken from one host population should be, on average, less virulent in hosts from other populations. Virulence in these novel hosts should, on average, decrease with decreasing genetic similarity between the host of origin and the novel host. 'Average' should be stressed here, since occasional highly virulent poorly reproducing parasites can be expected.

Box 2. Testing the Red Queen: does outbreeding and host genetic diversity hinder parasite adaptation?

(1) Parasites kept in monoclonal host cultures should evolve higher levels of virulence than parasites in multiclonal host populations. To avoid inevitable selection on host genotypes, the genetic composition and gene frequencies of the mixed host populations must be kept constant throughout the selection period. This can be done by continually reconstituting the host population from the same mixture of the same stocks. Without such replacement, selection for the least susceptible host genotype will occur and confound the result. The same replacement procedure must be done in the monoclonal host populations, to avoid selection on mutants and provide comparable handling and interference.
(2) Parasites kept in monoclonal host cultures should lose (reduce) their virulence when combined with other host clones, even if they were previously adapted to these clones.
(3) Parasites adapted to a monoclonal host population should express, on average, lower virulence in sexually outbred descendants of their hosts compared with inbred or asexually produced offspring. Variance of virulence and parasite success, however, should be high in the outbred offspring generation, and particularly high variance due to new homozygous combinations may become apparent in grand offspring and in later descendants from the outcrosses.

Box 3. Testing the Red Queen: do parasites mediate selection and polymorphism in natural host populations?

(1) Natural host populations that suffer intense parasite pressure should maintain higher levels of genetic variability than host populations without parasites. In clonal or cyclic parthenogenetic host populations, in which the whole host genome represents one linkage group, this effect can be investigated by correlating diversity of genetic markers with some measure of parasite selection pressure (e.g. mean parasite richness or abundance) across host populations. For sexually reproducing hosts, the allelic diversity of the defence loci can be studied (e.g. MHC haplotypes in vertebrates).
(2) In clonal and cyclic parthenogenetic host populations, linkage and selection by parasites are likely to produce genotype frequencies that differ between parasitized and uninfected hosts. In host–parasite systems where genes involved in defence are known (e.g. the MHC in vertebrates), the frequency of these genes can be studied directly.

Box 4. Testing the Red Queen: do parasites induce temporal changes in their host populations?

(1) Following prediction (2) in Box 3, if associations between parasites and host genotypes are detected and host generation time is short, parasite-mediated selection may be tested by re-sampling the same host population and monitoring changes in the frequency of the particular genotype (clonal markers or MHC genes, respectively).
(2) When parasite strains can be stored unchanged (e.g. by freezing of microbial pathogens), their re-introduction into the same host population many host generations later should reveal a change in their average virulence—most often a reduction in virulence. Due to ongoing host evolution, some originally highly virulent strains are likely to have become much less virulent.
(3) A similarity should be found between patterns of virulence in hosts over space and genetic distance [hosts vary spatially: see prediction (3) in Box 1] and changes in virulence when a genetically 'frozen' parasite is applied to the same local population over time [hosts vary temporally: see prediction (2) in Box 4].

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