

## Expanding the horizon: the Red Queen and potential alternatives

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**Abstract:** The Red Queen hypothesis (RQH) is one of the most widely accepted hypotheses explaining the persistence of sexual reproduction despite its costs. It posits that sexual species, compared with asexuals, are more adept at countering parasites, because their per-generation recombination rate is higher. Despite theoretical support, current empirical studies have failed to provide unanimous support. Here, we suggest that future tests of the RQH should more thoroughly elucidate its underlying assumptions and potential alternative hypotheses. While the RQH predicts that negative frequency-dependent selection shapes host–parasite interactions, differences between sexuals and asexuals are potentially important. Key assumptions about asexual species and their sexual close relatives include (i) ecological and behavioral traits are similar, (ii) among-individual genetic diversity is greater in sexuals than in asexuals, and (iii) within-individual genetic diversity is similar in asexuals and sexuals. We review current evidence for the RQH, highlight differences between asexual and sexual species and how those differences might translate into differential responses to parasite infections, and discuss how they can influence the results and interpretation of empirical studies. Considering differences between asexual and sexual species in future tests of the RQH will help to refine predictions and eliminate alternative hypotheses.

**Résumé :** L'hypothèse de la reine rouge (RQH) est l'une des hypothèses les plus généralement acceptées pour expliquer la persistance de la reproduction sexuelle malgré son coût. Elle suppose que les espèces sexuées, par comparaison aux asexuées, peuvent contrer leurs parasites plus efficacement parce que leur taux de recombinaison par génération est plus élevé. Malgré l'appui théorique à cette hypothèse, les études empiriques courantes ne fournissent pas de confirmation unanime. Nous proposons ici que les évaluations futures de la RQH devraient élucider plus attentivement les propositions sous-jacentes et considérer les hypothèses potentielles de rechange. Alors que la RQH prédit qu'une sélection négative dépendante de la fréquence affecte les interactions hôtes–parasites, les différences entre les sexués et les asexués peuvent s'avérer importantes. Les présuppositions principales faites au sujet des espèces asexuées et de leurs proches parents sexués sont que (i) leurs traits écologiques et comportementaux sont semblables, (ii) la diversité génétique parmi les individus est plus élevée chez les sexués que chez les asexués et (iii) la diversité génétique individuelle est la même chez les asexués et les sexués. Nous passons en revue les appuis actuels à la RQH, mettons en évidence les différences entre les espèces asexuées et sexuées, montrons comment ces différences peuvent mener à des réactions différentes à les infections parasitaires et discutons comment tout ceci peut influencer les résultats et l'interprétation des études empiriques. La prise en considération des différences entre les espèces asexuées et sexuées dans les évaluations futures de la RQH permettra de raffiner les prédictions et d'éliminer les hypothèses de rechange.

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### Introduction

Parasites are omnipresent in natural systems (Bush et al. 2001). The relationship between hosts and parasites is a prime example of antagonistic coevolution, whereby hosts refine their defense strategies against parasites and parasites in turn evolve counter-adaptations to those defense strategies. Variation in specific host–parasite interactions (i.e.,

variation within host species to be resistant against certain parasite genotypes and variation within parasite species to infect certain host genotypes) can result in time-lagged, negative frequency-dependent selection (Hamilton 1980), leading to rapid coevolutionary dynamics over short periods of time (Peters and Lively 1999). Typically, this coevolutionary interaction is depicted as an oscillating, cyclical process, but reality might be leading to more complex and

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**Table 1.** Testable prediction based on the Red Queen hypothesis.

Prediction	References
(1) Sexual reproduction should be favored when infection risk is high	Lively 1987; Hamilton et al. 1990
(2) Because of time-lagged frequency-dependent selection, recently common host genotypes should be most susceptible (also testable in purely sexual and purely asexual populations)	Bell 1982; Dybdahl and Lively 1998
(3) Given a lower genetic diversity in asexuals, higher parasite loads are expected in parthenogens, if closely related asexual and sexual forms coexist	Ladle 1992
(4) The relative frequency of host and parasite genotypes should vary over time (also testable in purely sexual and purely asexual populations)	Bell 1982; Seger and Hamilton 1988
(5) Parasites should be adapted to local host genotypes (also testable in purely sexual and purely asexual populations)	Lively 1989; Lively and Dybdahl 2000

less predictable dynamics. This idea is known as the Red Queen hypothesis (RQH),<sup>3</sup> and in its most general interpretation it has served as an explanation for the evolution and maintenance of genetic variation within host populations (Schmid-Hempel and Ebert 2003). For example, parasite diversity drives genetic polymorphisms at the major histocompatibility complex (MHC) in sticklebacks (Wegner et al. 2003) and cyprinid fishes (Simkova et al. 2006), and colonies of bumblebees having queens that mated multiply have less parasites (Baer and Schmid-Hempel 2001).

More specifically, the RQH is currently one of the most widely accepted hypotheses to explain the maintenance of sex and recombination (Jaenike 1978; Hamilton 1980; Bell 1982; Seger and Hamilton 1988; Hamilton et al. 1990), an unresolved paradox in evolutionary biology numerous theories have attempted to explain (Ladle 1992; Kondrashov 1993; West et al. 1999). All other things being equal (and this is a critical assumption), an asexually reproducing individual can propagate its genes with twice the rate of a sexual conspecific. Furthermore, a sexually reproducing individual passes on only half of its genes to the next generation, and successful genotypes are destroyed by recombination in every reproductive cycle. Although sex can accelerate the rate of evolution and in the long term may reduce the probability of extinction (Maynard Smith 1978; Ridley 2004), asexuality provides a short-term advantage that needs to be overcome by sexual reproduction. Thus, theoretically asexual lineages should be able to replace sexuals over short periods of time (Maynard Smith 1978; Lively and Lloyd 1990). This is a striking mismatch of theory and reality, because despite its twofold costs, sexual reproduction is omnipresent. The RQH attempts to explain this by arguing that recombination results in genetically diverse offspring that, contrary to the more uniform offspring of asexuals, are difficult, "moving" targets for parasites and diseases. Accordingly, the reduction of fitness through coevolving parasites and diseases in asexuals contributes to the short-term persistence of sexual reproduction

despite its costs. Henceforth, we focus solely on the RQH as a potential explanation for the maintenance of sex.

### Testing the Red Queen

Although mathematical models have provided theoretical support for the RQH (Hamilton et al. 1990; Agrawal 2006; but see Otto and Nuismer 2004), there is a current dearth of empirical studies (Wuethrich 1998). The RQH makes several predictions (Table 1; see also Ebert and Hamilton 1996), some of which are testable in purely asexual or purely sexual populations, whereas others require comparisons of closely related asexuals and sexuals that coexist in the same habitat. Studies on purely asexual and sexual populations provide knowledge on Red Queen dynamics that have important implications in the understanding of the RQH's role in the maintenance of sex. Although Red Queen cycles may be present in a given system (either sexual or asexual), the only way to estimate the degree to which parasites select for sex is the comparison of coexisting sexuals and asexuals.

Empirical studies testing the predictions of the RQH have shown inconsistent results (Table 2). Nonetheless, this hypothesis has entered the textbooks of evolutionary biology as a solution for the paradox of sex (Freeman and Herron 2001; Maynard Smith 2002; Ridley 2004). One possible reason for the inconsistent findings of previous tests of the RQH is that a fundamental assumption in all studies relying on the pairwise comparison of spatial and temporal patterns of parasitism between closely related sexual and asexual forms is not fulfilled: the assumption that apart from the reproductive mode all other things are equal.

### Asexuals are different

Ideally sexuals and asexuals compared to test the RQH should coexist ecologically, and only differ in their mode of reproduction. Implicitly, this is assumed by most studies. According to this basic assumption, the susceptibility to parasite infections of a given genotype, both in sexuals and in

<sup>3</sup>The Red Queen hypothesis was originally developed by Van Valen (1973) to account for the observation that in fossil records the extinction of a taxon is unrelated to its age. The hypothesis is a model of coevolution among species competing for resources in an "adaptive zone". If one species gains an advantage in exploiting the resource, a suitable response needs to evolve in the other taxa or they eventually become extinct. In such a model, species are constantly evolving, but relative fitness is not changing. The name of this hypothesis was inspired by Lewis Carroll's *Alice through the Looking Glass* as the Red Queen said to Alice: "Here you see, it takes all the running you can do, to keep in the same place." Since Van Valen (1973), the Red Queen has been applied to many ideas in evolutionary biology and related fields, e.g., to explain the coevolutionary arms race between hosts and their parasites (Schmid-Hempel and Ebert 2003), the role of parasites in the maintenance of sexual reproduction (Bell 1982), the evolutionary dynamics of predator-prey interactions (Dercote et al. 2006), or understanding lymphocyte survival (Freitas and Rocha 1997).

asexuals, is only dependent on Red Queen dynamics, i.e., the frequency of the genotype in the population (Bell 1982; Seger and Hamilton 1988). However, genetic (Gray and Gill 1993; Malo and Skamene 1994; Carton et al. 2005), ecological (Tinsley 1989; Reimchen and Nosil 2001), and behavioral (Moore 2002) factors also affect susceptibility of hosts to parasitism, although these have been largely ignored in the study of the RQH. These factors may potentially influence the observed patterns of parasitism making any interpretation of Red Queen mechanisms difficult. It often remains unclear if the causes of significant differences in parasitism between sexuals and asexual are in fact due to frequency-dependent selection (e.g., Hakoyama et al. 2001). Furthermore, the lack of differences in parasitism per se does not provide evidence that the Red Queen mechanisms are not at work (e.g., Tobler and Schlupp 2005). Here, we review current evidence for the RQH, highlight fundamental differences between asexuals and sexuals at three levels of organization (i.e., genome, individual, and population), and discuss how these differences may influence the results of empirical studies.

### Ecological and behavioral differences

Although coexisting in the same habitats, ecological differences can be found in some model systems, which potentially lead to differential exposure to infectious agents. Such differences have been shown to cause differential parasitism even within species. For example, female and male sticklebacks feed on different food items and as a consequence are parasitized by different parasites (Reimchen and Nosil 2001). Ecological differentiation has been reported between sexuals and asexuals. For example, in fishes of the genus *Poeciliopsis*, parthenogens were hypothesized to use resources underutilized by the coexisting sexual parental species (frozen niche variation model; Vrijenhoek 1979), and have different microhabitat use and foraging behaviors (Schenck and Vrijenhoek 1989). Currently, we remain ignorant about how ecological differences among the reproductive forms contribute to differences in parasitism, and if changes of ecological preferences in asexuals evolved to minimize the exposure to parasites.

Similarly, behavioral adaptations have been shown to reduce infection risk in many sexual species (Moore 2002). Consequently, behavioral counter-adaptations may also be expected in asexuals. Contrary to the sexual parental species, asexual Amazon mollies (*Poecilia formosa*) avoid males infected with black-spot disease (Tobler et al. 2006). Although this trematode-induced disease is not directly transmitted, secondary infections with fungi, bacteria, and viruses might be present in infected fish (Lane and Morris 2000), and drive the evolution and maintenance of the preference of asexuals (Tobler et al. 2006). In conclusion, differences in patterns of parasitism between asexuals and sexuals may be consistent with the RQH, but may also be explained by ecological or behavioral differentiation.

### Genetic diversity: a population perspective

Even more important than ecological and behavioral dif-

ferentiations, sexuals and closely related clonal forms often differ fundamentally in their genetic composition. A central assumption of the RQH is that rare genotypes have a selective advantage over common genotypes. Because of the effective lack of recombination, asexuals are assumed to be unable to generate new and rare genotypes (except through mutation). Consequently, genetic diversity should be lower in asexual than sexual populations. Intriguingly, mutation is but one source of genetic variation in asexuals. Here we consider the two most important evolutionary scenarios.

### Single vs. multiple origin of asexuals

If an asexual form is of single origin, the major source of genetic diversity within the asexual population is mutation; however, introgression was reported in some sperm-dependent asexual species (Schartl et al. 1995; Lampert et al. 2005; D'Souza et al. 2006). As a consequence of a single origin, the genetic diversity within populations of asexuals relative to the sexual progenitors is assumed to be low. Because of their twofold advantage, clones should become common within a short period of time and according to the RQH become susceptible to frequency-dependent selection through parasites. Single origin of parthenogens in metazoans seems to be an exception rather than a rule. The only documented case so far is the Amazon molly (*Poecilia formosa*; D. Möller et al., in preparation<sup>4</sup>), but the expected differences in parasitism compared with the coexisting sexual species could not be detected (Tobler and Schlupp 2005; Tobler et al. 2005; M. Tobler, unpublished data).

Most asexuals derived from multiple origins in space and time (e.g., Wetherington et al. 1989; Jokela et al. 2003), and continue to form de novo from their sexual progenitors through hybridization or polyploidization. The major source of genetic diversity within such asexual populations is not mutation, but the repeated evolution of asexuality. Thus, the genetic diversity within an asexual population is not necessarily significantly lower compared with the sexual progenitors, and multiclonal populations of parthenogens may be able to coexist with parasites in the long term (Dybdahl and Lively 1995).

### Incorporating immunogenetics

Given the differences in genetic diversity among asexuals, future tests of the RQH should be accompanied by monitoring the genetic diversity of host populations. This is relatively easy in purely clonal populations, since loci involved in the host-parasite interaction are genetically linked to the marker genes for the identification of clonal diversity (e.g., Dybdahl and Lively 1998; but for ameiotic recombination in clonal lineages see Omilian et al. 2006). However, this is not possible if asexuals coexist with closely related sexuals and share a common pool of parasites.

In this case, the genetic diversity of clonal and sexual lineages must not be compared at any random locus but at loci relevant for the specific host-parasite interaction. Whereas the whole genome is one large hereditary unit in asexuals, the recombination in sexuals allows for independent rates of evolution in different parts of the genome. In the clonal

<sup>4</sup>D. Möller, J. Parzefall, M. Schartl, and I. Schlupp. Eve of Amazons. In preparation.

**Table 2.** Empirical tests of the Red Queen hypothesis.

Study system	Difference between reproductive modes	Prediction	Support	References
Crustaceans of the genus <i>Daphnia</i> O.F. Müller, 1785 parasitized by microparasites	—	1	No	Killick et al. 2008
Fish of the genus <i>Phoxinus</i> Rafinesque, 1820 parasitized by a monogenean trematode	Asexuals are hybrids	3	Yes	Mee and Rowe 2006
Fish of the genus <i>Poecilia</i> Bloch and Schneider, 1801 parasitized by various species	Asexuals are hybrids	3	No	Tobler and Schlupp 2005
Fish of the genus <i>Poeciliopsis</i> Regan, 1913 parasitized by a trematode	Asexuals are hybrids	2	No	Weeks 1996
Fish of the genus <i>Poeciliopsis</i> parasitized by a trematode	Asexuals are hybrids	3	Yes	Lively et al. 1990
Geckos of the genera <i>Hemidactylus</i> Gray, 1825 and <i>Lepidodactylus</i> Fitzinger, 1843 parasitized by mites	Asexuals are hybrids	3	No	Brown et al. 1995; Hanley et al. 1995
Geckos <i>Heteronotia binoei</i> (Gray, 1845) parasitized by mites	Asexuals are polyploids	3	Yes	Moritz et al. 1991
Psychid moths parasitized by hymenopteran parasitoids	Asexuals are polyploid	1	Yes	Kumpulainen et al. 2004
The bryozoan <i>Cristatella mucedo</i> Cuvier, 1798 parasitized by myxozoans	—	2	No	Vernon et al. 1996
The flatworm <i>Schmidtea polychroa</i> (Schmidt, 1861) parasitized by an amoeboid	Asexuals are triploid	3	Yes	Michiels et al. 2001
The freshwater fish <i>Carassius auratus</i> (L., 1758) parasitized by a trematode	Asexuals are polyploid	3	Yes	Hakoyama et al. 2001
The freshwater snail <i>Campeloma decisum</i> (Say, 1817) parasitized by a trematode	Asexuals are either autodiploids or polyploids	3	Yes	Johnson 1994, 2000
The freshwater snail <i>Melanoides tuberculata</i> parasitized by a trematode	Asexuals are polyploid	1	No	Ben-Ami and Heller 2005
The freshwater snail <i>Potamopyrgus antipodarum</i> (J.E. Gray, 1853) parasitized by a trematode	Asexuals are triploid	1	Yes	Lively 1987; Lively and Jokela 2002; Jokela et al. 2003
The freshwater snail <i>Potamopyrgus antipodarum</i> parasitized by a trematode	Asexuals are triploid	2	Yes	Dybdahl and Lively 1998
The freshwater snail <i>Potamopyrgus antipodarum</i> parasitized by a trematode	Asexuals are triploid	4	Yes	Dybdahl and Lively 1998
The freshwater snail <i>Potamopyrgus antipodarum</i> parasitized by a trematode	Asexuals are triploid	5	Yes	Lively 1989; Lively and Dybdahl 2000

subpopulation, frequency-dependent changes can be revealed by looking for changes in clonal frequencies; however, in sexuals, such changes will only be detectable in changes of allele frequencies at loci involved in the host–parasite interaction.

While our understanding of invertebrate immune systems is still fragmentary, vertebrate immune defense is well understood (Janeway et al. 1999). The vertebrate immune system relies on two lines of defense: innate immunity and acquired immunity. Innate immunity is an efficient first protection against many pathogens; however, these innate reactions are not specific (Janeway et al. 1999). Adaptive immunity, on the other hand, is highly specific and antibody-based. Antigen-presenting cells bind nonself peptides (e.g., peptides from the proteins of parasites) to receptors of the major histocompatibility complex (MHC) and interact with T-lymphocytes. These cells activate antibody producing B-lymphocytes (Janeway et al. 1999; Penn and Potts 1999).

The first step in a successful immune reaction is the recognition of parasites. In this process, genes in the MHC play a key role. MHC genes are highly polymorphic, and it was hypothesized that parasite-driven selection pressure maintains the high genetic diversity at MHC loci (Doherty and Zinkernagel 1975; Apanius et al. 1997; Penn and Potts 1999). Since the product of the MHC genes, the MHC receptors, interact directly with the peptides of pathogens, the genes in the MHC are excellent candidate genes for the detection of parasite-induced frequency-dependent processes in vertebrates.

Thus, future tests of the RQH should include long-term (over several host generations) field studies to assay frequency-dependent changes at loci relevant in host–parasite interactions. Such simultaneous examination of parasitism patterns in coexisting asexual and sexual populations can test the idea that parasites are driving these time-lagged, frequency-dependent processes. Finally, correlative field data should be supported by challenge experiments in controlled laboratory studies (see Dybdahl and Lively 1998). In challenge experiments, hosts are exposed to pathogens and host performance is measured using a variety of indirect and direct tests, such as immunoassays or directly measuring mortality. For tests of the RQH, locally common and rare host genotypes should be exposed to parasite isolates (Dybdahl and Lively 1998). Under standardized experimental conditions, recently common clones are expected to be most susceptible.

### Genetic diversity: an individual perspective

There are at least two alternative and potentially important factors causing differential susceptibility of asexuals and sexuals at the individual level: (1) accumulation of deleterious mutations and (2) the mode of origin, which influences the genetic diversity within individuals. A closer look at these factors illustrates problems in the interpretation of data concerning the distribution of parasites in asexual and sexual forms because multiple hypotheses predict a higher parasite load in asexuals.

### Mutation accumulation

Asexual lineages are hypothesized to accumulate deleteri-

ous mutations over the course of time (Muller 1964; Kondrashov 1988; Chao 1990; Vorburger 2001). The accumulation of mutations in genes relevant for immune defense may lead to a deterioration of immune function. For example, asexual *Carassius* have lower phagocyte activity than their sexual relatives (Hakoyama et al. 2001). Similarly, bacteria with high mutation loads have lower resistance to phage infections and suffer from higher fitness costs when evolving resistance (Buckling et al. 2006). As in the RQH, an impaired immune function owing to accumulation of mutations in asexual lineages is predicted to result in higher parasite loads compared with coexisting sexual relatives. Consequently, the susceptibility to parasites of a given clonal lineage is a function of time, where relatively young clones have an intact immune system and similar parasite loads as sexuals. Only older clones suffer from higher parasite loads, which ultimately may cause the extinction of that clonal lineage. A pattern consistent with this hypothesis was found by Neiman et al. (2005) in the snail *Potamopyrgus antipodarum*. Here older asexual lineages were only found at sites where parasite prevalence was low but were absent in parasite-rich habitats.

Therefore, finding higher parasite loads in the asexual lineage of two coexisting asexual and sexual relatives is not only consistent with predictions of at least two different hypotheses, the RQH and the mutation accumulation hypothesis, but there may be an interactive effect in that the presence of mutations may slow down the coevolutionary dynamics expected by the RQH (Buckling et al. 2006).

### Effects of hybridization and polyploidization

Most asexual metazoans originated through hybridization or auto-polyploidization (Dawley 1989). The mode of origin has been shown to influence fitness components in asexuals (Johnson 2005) and it may also have consequences for the immune function of asexuals. However, theoretical models on the effects of hybridization and polyploidization on immune function provide no clear predictions.

Regarding hybridization, three basic quantitative models of hybrid resistance have been proposed for animal and plant model systems (Fritz et al. 1999; Mouliia 1999): (1) an additive model in which hybrid resistance is intermediate to that of the parental species, (2) dominance and partial dominance models in which hybrid resistance resembles the resistance of one parental species (dominance of susceptibility or dominance of resistance), and (3) overdominance models with hybrid resistance significantly lower (overdominance for susceptibility, outbreeding depression, hybrid susceptibility) or higher (heterosis, overdominance for resistance, hybrid resistance) than that of parental species. Patterns of disease resistance predicted by the above models have been documented from natural (Fritz et al. 1999; Mouliia 1999) and artificial (Fjalestad et al. 1993) hybrids.

Similarly, polyploidization was hypothesized to have favorable and adverse effects on disease resistance. Additional sets of chromosomes in polyploid asexuals lead to increased genetic diversity within an individual, which may have a positive effect on the resistance to parasites (LaPatra et al. 1996; Nuismer and Otto 2004; Osnas and Lively 2006). Increased ploidy may have an adverse effect on the immune system, because polyploidization can result in an

increased cell size, which can lead to a decreased number of cells in the body and changes in cell shape (Fankhauser 1945; Otto and Whitton 2000). This will particularly affect physiological processes in which cell size and cell number may play a crucial role (like immune function; Hakoyama et al. 2001). Empirically, lower levels of disease resistance have been documented in natural (Hakoyama et al. 2001) and artificial (Langston et al. 2001) polyploids.

In essence, it is very hard to predict the level of genetic immunity in asexuals at their origin from a sexual ancestor. Furthermore, the predictions on how mutation accumulation, hybridization, and polyploidization affect the parasite loads in coexisting sexual and asexual lineages can be the same as in the RQH. In systems where asexuals are continuously emerging from sexuals, a short-term persistence of individual clonal lineages with comparatively low levels of immunity might be possible. Parasites may certainly play a role in the decline and extinction of clonal lineages, which would be reflected in the pattern of parasitism, but instead of frequency-dependent selection, disadvantages arising from hybridization, polyploidization, or accumulation of deleterious mutations could also be the driving forces.

Likely, only asexuals starting out with the same or even a higher immune function as their coexisting sexual relatives can persist in the long term. The initial level of immune function likely will have an influence on the level of immune function that can be observed in present asexuals after generations of parthenogenetic reproduction and clonal selection. The influence of Red Queen mechanisms can be expected to erode the potential initial advantage of asexuals by the quickly coevolving parasites (Osnas and Lively 2006). Nonetheless, improved immunocompetence to non-coevolved parasites might be advantageous in the long term, since besides the few highly virulent pathogens driving a system there might be a pool of slower or non-coevolving pathogens that still have fitness consequences for their hosts.

Only challenge experiments can reveal how these factors and processes influence immunocompetence of recent asexuals. Further, such experiments will help to identify the consequences (i.e., frequency-dependent dynamics) of differences in immunocompetence between sexuals and asexuals. For this purpose, asexuals and sexuals should not only be exposed to coevolved parasite isolates from natural populations, since possible differences in immune response cannot be attributed only to the immune system of the host, but also to adaptations of the pathogen to certain host genotypes (see Lively and Dybdahl 2000). Such experiments should further be accompanied by immunogenetic and genetic analyses to estimate the influence of differences in within host genetic variation between sexuals and asexuals.

### Differences in resource allocation and life-history strategies

A fundamental assumption of the RQH is that a host's susceptibility is dependent on the interaction of host and parasite genotypes. However, cases in which the outcome of a host-parasite interaction is specifically determined by host and parasite genotype (allele models; Schmid-Hempel and Ebert 2003) are probably rare (but see Thompson and Burdon 1992; Schmid-Hempel et al. 1999; Carius et al.

2001). In theory, immune response can be entirely non-specific and effective against all relevant parasites. Non-genetic factors such as host condition play an important role in disease resistance, because using the immune system is associated with significant costs (Sheldon and Verhulst 1996; Zuk et al. 1996; Gemmill and Read 1998; Schmid-Hempel 2003; Schmid-Hempel and Ebert 2003).

Immune defense is not an all or nothing process, but different branches of the immune system that are associated with different levels of costs and can be regulated independently (Janeway et al. 1999). Constitutive responses, for example, are always present and capable of defense without previous contact, whereas induced responses are only activated after a parasite or disease vector has been recognized. Consequently, immune response to a given pathogen can vary in time, duration, specificity, and intensity (Schmid-Hempel 2003). The actual immune response is not only dependent on the genetic background of an individual, but the availability of resources (e.g., energy, proteins, and other specific nutrients to build up the effectors of the immune response) has an influence especially on its duration and intensity (Sheldon and Verhulst 1996; Zuk and Stoehr 2002).

### Hyper-resistant asexuals?

In theory, hyper-resistant parthenogens may not only arise through generations of clonal selection, but we hypothesize that asexuals facing a genetic disadvantage (e.g., through Red Queen processes) may be selected for investing more resources in immune defense. Taking into account the two-fold advantage of asexual reproduction, the additional investment into immune defense could be substantial. Asexuals could invest more in constitutive responses or generally start the response more rapidly, maintain it for a longer period of time and with a higher intensity. Consequently, the higher the influence of nongenetic factors for minimizing fitness reductions owing to parasitism, the more patterns of parasitism predicted by the RQH may actually be abolished.

More importantly, the costs of an elevated investment into immune defense would eventually precipitate trade-offs of other life-history traits (e.g., growth, age of maturity, reproductive output). Thus, the ultimate reduction of the twofold advantage through changes in resource allocation and life-history strategies could contribute to the maintenance of sexual reproduction.

### Concluding remarks

Although the theory that coevolving parasites select for sex is highly intuitive, empirical tests have provided equivocal results. Unfortunately, basic assumptions of the RQH are often only partially fulfilled, and in most systems studied so far, this has so far not fully been addressed. A notable exception is the work of C.M. Lively and co-workers studying the RQH in the freshwater snail *Potamopyrgus antipodarum* (for a review see Jokela et al. 2003).

The basic assumptions do not only include the genetics and ecology of the hosts that were discussed here, but also genetics and ecology of the involved parasites. Parasites must have a considerable virulence to yield an advantage to

sexuals that allow them to overcome the twofold advantage of asexuals (Howard and Lively 1998; Michiels et al. 2001; but see Agrawal 2006). Most studies so far have focused on a single macroparasite species with comparatively long generation times, in which the actual damage to the host is unknown. Rather than choosing a parasite a priori, future approaches should attempt to survey multiple parasites within a host system to identify species that go through coevolutionary cycles with their hosts and have a high-enough virulence. Bacteria, viruses, and other microparasites should be taken into consideration, since they are abundant pathogens and are known to have the potential to be highly virulent (Dronamraju 2004).

Considering differences (at the levels of genes, individuals, and populations) between asexuals and sexuals in future tests of the RQH will help to refine predictions and eliminate alternative hypotheses. This necessitates the long-term examination of hosts and their parasites in natural populations, accompanied by studies of the genetics, immune biology, life history, and ecology of the involved species. Theoretically, there are various mechanisms by which parasites can select for sex. Considering these factors will help to determine the most important mechanisms, as well as their interactions with other potential selective forces, that have been proposed to select for sex (West et al. 1999).

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## References

- Agrawal, A. 2006. Similarity selection and the evolution of sex: revisiting the Red Queen. *PLoS Biol.* **4**: e265. doi:10.1371/journal.pbio.0040265. PMID:16869713.
- Apanius, V., Penn, D., Slev, P., Ruff, L.R., and Potts, W.K. 1997. The nature of selection on the major histocompatibility complex. *Crit. Rev. Immunol.* **17**: 179–224. PMID:9094452.
- Baer, B., and Schmid-Hempel, P. 2001. Unexpected consequences of polyandry for parasitism and fitness in the bumblebee, *Bombus terrestris*. *Evolution*, **55**: 1639–1643. PMID:11580023.
- Bell, G. 1982. The masterpiece of nature, the evolution and genetics of sexuality. University of California Press, Berkeley.
- Ben-Ami, F., and Heller, J. 2005. Spatial and temporal patterns of parthenogenesis and parasitism in the freshwater snail *Melanooides tuberculata*. *J. Evol. Biol.* **18**: 138–146. doi:10.1111/j.1420-9101.2004.00791.x. PMID:15669970.
- Brown, S.G., Kwan, S., and Shero, S. 1995. The parasitic theory of sexual reproduction: parasitism in unisexual and bisexual geckos. *Proc. R. Soc. Lond. B Biol. Sci.* **260**: 317–320. doi:10.1098/rspb.1995.0098.
- Buckling, A., Wei, Y., Massey, R.C., Brockhurst, M.A., and Hochberg, M.E. 2006. Antagonistic co-evolution with parasites increases the cost of host deleterious mutations. *Proc. R. Soc. Lond. B Biol. Sci.* **273**: 45–49. doi:10.1098/rspb.2005.3279.
- Bush, A.O., Fernández, J.C., Esch, G.W., and Seed, J.R. 2001. Parasitism: the diversity and ecology of animal parasites. Cambridge University Press, Cambridge.
- Carius, H., Little, T., and Ebert, D. 2001. Genetic variation in a host–parasite associations: potential for co-evolution and frequency-dependent selection. *Evolution*, **55**: 1136–1145. PMID:11475049.
- Carton, Y., Nappi, A., and Poirie, M. 2005. Genetics of anti-parasite resistance in invertebrates. *Dev. Comp. Immunol.* **29**: 9–32. doi:10.1016/j.dci.2004.05.004. PMID:15325520.
- Chao, L. 1990. Fitness of RNA virus decreased by Muller's ratchet. *Nature (London)*, **348**: 454–455. doi:10.1038/348454a0. PMID:2247152.
- Dawley, R.M. 1989. An introduction to unisexual vertebrates. In *Evolution and ecology of unisexual vertebrates. Edited by R.M. Dawley and J.P. Bogart.* Bull. No. 466, New York State Museum, New York. pp. 1–18.
- Dercole, F., Ferriere, R., Gragnani, A., and Rinaldi, S. 2006. Co-evolution of slow-fast populations: evolutionary sliding, evolutionary pseudo-equilibria and complex Red Queen dynamics. *Proc. R. Soc. Lond. B Biol. Sci.* **273**: 983–990. doi:10.1098/rspb.2005.3398.
- Doherty, P., and Zinkernagel, R. 1975. Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. *Nature (London)*, **256**: 50–52. doi:10.1038/256050a0. PMID:1079575.
- Dronamraju, K. 2004. Infectious disease and host–pathogen evolution. Cambridge University Press, Cambridge.
- D'Souza, T.G., Schulte, R.D., Schulenburg, H., and Michiels, N.K. 2006. Paternal inheritance in parthenogenetic forms of the planarian *Schmidtea polychroa*. *Heredity*, **97**: 97–101. doi:10.1038/sj.hdy.6800841. PMID:16721392.
- Dybdahl, M.F., and Lively, C.M. 1995. Diverse, endemic and polyphyletic clones in mixed populations of a fresh-water snail (*Potamopyrgus antipodarum*). *J. Evol. Biol.* **8**: 385–398. doi:10.1046/j.1420-9101.1995.8030385.x.
- Dybdahl, M.F., and Lively, C.M. 1998. Host–parasite co-evolution: evidence for rare advantage and time-lagged selection in a natural population. *Evolution*, **52**: 1057–1066. doi:10.2307/2411236.
- Ebert, D., and Hamilton, W.D. 1996. Sex against virulence: the co-evolution of parasitic diseases. *Trends Ecol. Evol.* **11**: 79–82. doi:10.1016/0169-5347(96)81047-0.
- Fjalestad, K.T., Gjedrem, T., and Gierde, B. 1993. Genetic improvement of disease resistance in fish: an overview. *Aquaculture*, **111**: 65–74. doi:10.1016/0044-8486(93)90025-T.
- Fankhauser, G. 1945. Maintenance of normal structure in heteroploid salamander larvae, through compensation of changes in cell size by adjustments of cell number and cell shape. *J. Exp. Zool.* **100**: 445–455. doi:10.1002/jez.1401000310.
- Freeman, S., and Herron, J.C. 2001. *Evolutionary analysis.* 2nd ed. Prentice-Hall Inc., Upper Saddle River, N.J.
- Freitas, A., and Rocha, B. 1997. Lymphocyte survival: a Red Queen hypothesis. *Science (Washington, D.C.)*, **277**: 1950. doi:10.1126/science.277.5334.1950. PMID:9333949.
- Fritz, R.S., Moulia, C., and Newcombe, G. 1999. Resistance of hybrid plants and animals to herbivores, pathogens and parasites. *Annu. Rev. Ecol. Syst.* **30**: 565–591. doi:10.1146/annurev.ecolsys.30.1.565.
- Gemmill, A.W., and Read, A.F. 1998. Counting the cost of disease resistance. *Trends Ecol. Evol.* **13**: 8–9. doi:10.1016/S0169-5347(97)01240-8.
- Gray, G., and Gill, H. 1993. Host genes, parasites and parasitic infections. *Int. J. Parasitol.* **23**: 485–494. doi:10.1016/0020-7519(93)90037-Y. PMID:8354600.

- Hakoyama, H., Nishimura, T., Matsubara, N., and Iguchi, K. 2001. Difference in parasite load and nonspecific immune reaction between sexual and gynogenetic forms of *Carassius auratus*. *Biol. J. Linn. Soc.* **72**: 401–407.
- Hamilton, W.D. 1980. Sex versus non-sex versus parasite. *Oikos*, **35**: 282–290. doi:10.2307/3544435.
- Hamilton, W.D., Axelrod, R., and Tanese, R. 1990. Sexual reproduction as an adaptation to resist parasites (a review). *Proc. Natl. Acad. Sci. U.S.A.* **87**: 3566–3573. doi:10.1073/pnas.87.9.3566. PMID:2185476.
- Hanley, K.A., Fisher, R.N., and Case, T.J. 1995. Lower mite infestations in an asexual gecko compared with its sexual ancestors. *Evolution*, **49**: 418–426. doi:10.2307/2410266.
- Howard, R.S., and Lively, C.M. 1998. The maintenance of sex by parasitism and mutation accumulation under epistatic fitness functions. *Evolution*, **52**: 604–610. doi:10.2307/2411094.
- Jaenike, J. 1978. A hypothesis to account for the maintenance of sex within population. *Evol. Theory*, **3**: 191–194.
- Janeway, C.A., Travers, P., Walport, M., and Capra, J.D. 1999. Immunobiology: the immune system in health and disease. Current Biology Publications, London.
- Johnson, S. 2005. Mode of origin differentially influences the fitness of parthenogenetic freshwater snails. *Proc. R. Soc. Lond. B Biol. Sci.* **272**: 2149–2153. doi:10.1098/rspb.2005.3208.
- Johnson, S.G. 1994. Parasitism, reproductive assurance and the evolution of reproductive mode in a freshwater snail. *Proc. R. Soc. Lond. B Biol. Sci.* **255**: 209–213. doi:10.1098/rspb.1994.0030.
- Johnson, S.G. 2000. Population structure, parasitism, and survivorship of sexual and autodiploid parthenogenetic *Campeloma limum*. *Evolution*, **54**: 167–175. PMID:10937193.
- Jokela, J., Lively, C.M., Dybdahl, M.F., and Fox, J.A. 2003. Genetic variation in sexual and clonal lineages of a freshwater snail. *Biol. J. Linn. Soc.* **79**: 165–181. doi:10.1046/j.1095-8312.2003.00181.x.
- Killick, S.C., Obbard, D.J., West, S.A., and Little, T.J. 2008. Parasitism and breeding system variation in North American populations of *Daphnia pulex*. *Ecol. Res.* **23**: 235–240. doi:10.1007/s11284-007-0368-x.
- Kondrashov, A.S. 1988. Deleterious mutations and the evolution of sexual reproduction. *Nature (London)*, **336**: 435–441. doi:10.1038/336435a0. PMID:3057385.
- Kondrashov, A.S. 1993. Classification of hypotheses on the advantage of amphimixis. *J. Hered.* **84**: 372–387. PMID:8409359.
- Kumpulainen, T., Grapputo, A., and Mappes, J. 2004. Parasites and sexual reproduction in psychid moths. *Evolution*, **58**: 1511–1520. PMID:15341153.
- Ladle, R.J. 1992. Parasites and sex: catching the Red Queen. *Trends Ecol. Evol.* **7**: 405–408. doi:10.1016/0169-5347(92)90021-3.
- Lampert, K.P., Lamatsch, D.K., Epplen, J.T., and Scharf, M. 2005. Evidence for a monophyletic origin of triploid clones of the Amazon molly, *Poecilia formosa*. *Evolution*, **59**: 881–889. PMID:15926697.
- Lane, R.L., and Morris, J.E. 2000. Biology, prevention, and effects of common grubs (digenetic trematodes) in freshwater fish. *Tech. Bull. Ser. Iowa State Univ.* **115**: 1–6.
- Langston, A.L., Johnstone, R., and Ellis, A.E. 2001. The kinetics of the hypoferremic response and changes in levels of alternative complement activity in diploid and triploid Atlantic salmon, following injection of lipopolysaccharides. *Fish Shellfish Immunol.* **11**: 333–345. doi:10.1006/fsim.2000.0319. PMID:11417720.
- LaPatra, S., Lauda, K., Jones, G., Shewmaker, W., Groff, J., and Routledge, D. 1996. Susceptibility and humoral response of brown trout x lake trout hybrids to infectious hematopoietic necrosis virus: a model for examining disease resistance mechanisms. *Aquaculture*, **146**: 179–188. doi:10.1016/S0044-8486(96)01381-6.
- Lively, C.M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism. *Nature (London)*, **328**: 519–521. doi:10.1038/328519a0.
- Lively, C.M. 1989. Adaptation by a parasitic trematode to local populations of its snail host. *Evolution*, **43**: 1663–1671. doi:10.2307/2409382.
- Lively, C.M., and Dybdahl, M.F. 2000. Parasite adaptation to locally common host genotypes. *Nature (London)*, **405**: 679–681. doi:10.1038/35015069. PMID:10864323.
- Lively, C.M., and Jokela, J. 2002. Temporal and spatial distributions of parasites and sex in a freshwater snail. *Evol. Ecol. Res.* **4**: 219–226.
- Lively, C.M., and Lloyd, D.G. 1990. The cost of biparental sex under individual selection. *Am. Nat.* **135**: 489–500. doi:10.1086/285058.
- Lively, C.M., Craddock, C., and Vrijenhoek, R.C. 1990. Red Queen hypothesis supported by parasitism in sexual and clonal fish. *Nature (London)*, **344**: 864–867. doi:10.1038/344864a0.
- Maynard Smith, J. 1978. The evolution of sex. Cambridge University Press, Cambridge.
- Maynard Smith, J. 2002. Evolutionary genetics. Oxford University Press, Oxford.
- Mee, J.A., and Rowe, L. 2006. A comparison of parasite loads on asexual and sexual *Phoxinus* (Pisces: Cyprinidae). *Can. J. Zool.* **84**: 808–816. doi:10.1139/Z06-064.
- Malo, D., and Skamene, E. 1994. Genetic control of host resistance to infection. *Trends Genet.* **10**: 365–371. doi:10.1016/0168-9525(94)90133-3. PMID:7985241.
- Michiels, N.K., Beukeboom, L.W., Pongratz, N., and Zeitlinger, J. 2001. Parthenogenetic flatworms have more symbionts than their coexisting, sexual conspecifics, but does this support the Red Queen? *J. Evol. Biol.* **14**: 110–119. doi:10.1046/j.1420-9101.2001.00249.x.
- Moore, J. 2002. Parasites and the behaviour of animals. Oxford University Press, Oxford.
- Moritz, C., McCallum, H., Donnellan, S., and Roberts, J.D. 1991. Parasite loads in a parthenogenetic and sexual lizards (*Heteronotia binoei*): support for the Red Queen hypothesis. *Proc. R. Soc. Lond. B Biol. Sci.* **244**: 145–149. doi:10.1098/rspb.1991.0063.
- Moulija, C. 1999. Parasitism of plant and animal hybrids: Are facts and fates the same? *Ecology*, **80**: 392–406.
- Muller, H.J. 1964. The relation of recombination to mutational advance. *Mutat. Res.* **1**: 2–9.
- Neiman, M., Jokela, J., and Lively, C.M. 2005. Variation in asexual lineage age in *Potamopyrgus antipodarum*, a New Zealand snail. *Evolution*, **59**: 1945–1952. PMID:16261732.
- Nuismer, S.L., and Otto, S.P. 2004. Host–parasite interactions and the evolution of ploidy. *Proc. Natl. Acad. Sci. U.S.A.* **101**: 11036–11039. doi:10.1073/pnas.0403151101. PMID:15252199.
- Omilian, A.R., Cristescu, M.E., Dudycha, J.L., and Lynch, M. 2006. Aneuploid recombination in asexual lineages of *Daphnia*. *Proc. Natl. Acad. Sci. U.S.A.* **103**: 18638–18643. doi:10.1073/pnas.0606435103. PMID:17121990.
- Osnas, E.E., and Lively, C.M. 2006. Host ploidy, parasitism and immune defense in a co-evolutionary snail–trematode system. *J. Evol. Biol.* **19**: 42–48. doi:10.1111/j.1420-9101.2005.00994.x. PMID:16405575.
- Otto, S., and Nuismer, S. 2004. Species interactions and the evolution of sex. *Science (Washington, D.C.)*, **304**: 1018–1020. doi:10.1126/science.1094072. PMID:15143283.

- Otto, S., and Whitton, J. 2000. Polyploid incidence and evolution. *Annu. Rev. Genet.* **34**: 401–437. doi:10.1146/annurev.genet.34.1.401. PMID:11092833.
- Penn, D.J., and Potts, W.K. 1999. The evolution of mating preferences and major histocompatibility complex genes. *Am. Nat.* **153**: 145–164. doi:10.1086/303166.
- Peters, A.D., and Lively, C.M. 1999. The Red Queen and fluctuating epistasis: a population genetic analysis of antagonistic co-evolution. *Am. Nat.* **154**: 393–405. doi:10.1086/303247. PMID:10523486.
- Reimchen, T.E., and Nosil, P. 2001. Ecological causes of sex-biased parasitism in threespined sticklebacks. *Biol. J. Linn. Soc.* **73**: 51–63.
- Ridley, M. 2004. *Evolution*. Blackwell Science, Oxford.
- Schartl, M., Nanda, I., Schlupp, I., Wilde, B., Epplen, J.T., Schmid, I., and Parzefall, J. 1995. Incorporation of subgenomic amounts of DNA as compensation for mutational load in a gynogenetic fish. *Nature (London)*, **373**: 68–71. doi:10.1038/373068a0.
- Schenck, R.A., and Vrijenhoek, R.C. 1989. Habitat selection and feeding behavior of sexual and clonal *Poeciliopsis*. In *The ecology and evolution of unisexual vertebrates*. Edited by R.M. Dawley and J.P. Bogart. Bulletin No. 466, New York State Museum, Albany. pp. 39–48.
- Schmid-Hempel, P. 2003. Variation in immune defense as a question of evolutionary ecology. *Proc. R. Soc. Lond. B Biol. Sci.* **270**: 357–366. doi:10.1098/rspb.2002.2265.
- Schmid-Hempel, P., and Ebert, D. 2003. On the evolutionary ecology of specific immune defence. *Trends Ecol. Evol.* **18**: 27–32. doi:10.1016/S0169-5347(02)00013-7.
- Schmid-Hempel, P., Pühr, K., Kruger, N., Reber, C., and Schmid-Hempel, R. 1999. Dynamic and genetic consequences of variation in horizontal transmission for a microparasite infection. *Evolution*, **53**: 426–434. doi:10.2307/2640779.
- Seger, J., and Hamilton, W.D. 1988. Parasites and sex. In *The evolution of sex*. Edited by R.E. Michod and B.R. Levin. Sinauer Associates, Inc., Sunderland, Mass. pp. 176–193.
- Sheldon, B.C., and Verhulst, S. 1996. Ecological immunology: costly parasite defenses and trade-offs in evolutionary ecology. *Trends Ecol. Evol.* **11**: 317–321. doi:10.1016/0169-5347(96)10039-2.
- Simkova, A., Ottova, E., and Morand, S. 2006. MHC variability, life-traits and parasite diversity of European cyprinid fish. *Evol. Ecol.* **20**: 465–477.
- Thompson, J.N., and Burdon, J.J. 1992. Gene-for-gene co-evolution between plants and parasites. *Nature (London)*, **360**: 121–125. doi:10.1038/360121a0.
- Tinsley, R. 1989. The effects of host sex on transmission success. *Parasitol. Today*, **5**: 190–195. doi:10.1016/0169-4758(89)90144-0. PMID:15463210.
- Tobler, M., and Schlupp, I. 2005. Parasites in sexual and asexual molly species of the genus *Poecilia* (Poeciliidae, Teleostei): a case for the Red Queen? *Biol. Lett.* **1**: 166–168. doi:10.1098/rsbl.2005.0305. PMID:17148156.
- Tobler, M., Wahli, T., and Schlupp, I. 2005. Comparison of parasite communities in native and introduced populations of sexual and asexual mollies of the genus *Poecilia*. *J. Fish Biol.* **67**: 1072–1082. doi:10.1111/j.0022-1112.2005.00810.x.
- Tobler, M., Plath, M., Burmeister, H., and Schlupp, I. 2006. Black spots and female association preferences in a sexual/asexual mating complex (*Poecilia*, Poeciliidae, Teleostei). *Behav. Ecol. Sociobiol.* **60**: 159–165. doi:10.1007/s00265-005-0152-2.
- Van Valen, L. 1973. A new evolutionary law. *Evol. Theory*, **1**: 1–30.
- Vernon, J.G., Okamura, B., Jones, C.S., and Noble, L.R. 1996. Temporal patterns of clonality and parasitism in a population of freshwater bryozoans. *Proc. R. Soc. Lond. B Biol. Sci.* **263**: 1313–1318. doi:10.1098/rspb.1996.0192.
- Vorburger, C. 2001. Fixation of deleterious mutations in clonal lineages: evidence from hybridogenetic frogs. *Evolution*, **55**: 2319–2332. PMID:11794790.
- Vrijenhoek, R.C. 1979. Factors affecting clonal diversity and co-existence. *Am. Zool.* **19**: 787–789.
- Weeks, S.C. 1996. A re-evaluation of the Red Queen model for the maintenance of sex in a clonal-sexual fish complex (*Poeciliopsis*). *Can. J. Fish. Aquat. Sci.* **53**: 1157–1164. doi:10.1139/cjfas-53-5-1157.
- Wegner, K.M., Reusch, T.B.H., and Kalbe, M. 2003. Multiple parasites are driving major histocompatibility complex polymorphism in the wild. *J. Evol. Biol.* **16**: 224–232. doi:10.1046/j.1420-9101.2003.00519.x. PMID:14635861.
- West, S.A., Lively, C.M., and Read, A.F. 1999. A pluralist approach to sex and recombination. *J. Evol. Biol.* **12**: 1003–1012. doi:10.1046/j.1420-9101.1999.00119.x.
- Wetherington, J.D., Schenck, R.A., and Vrijenhoek, R.C. 1989. The origins and ecological success of unisexual *Poeciliopsis*: the frozen niche-variation model. In *Ecology and evolution of live-bearing fishes (Poeciliidae)*. Edited by G.K. Meffe and F.F. Snelson. Prentice-Hall Inc., Englewood Cliffs, N.J. pp. 259–275.
- Wuethrich, B. 1998. Why sex? Putting theory to the test. *Science (Washington, D.C.)*, **281**: 1980–1982. doi:10.1126/science.281.5385.1980. PMID:9767049.
- Zuk, M., and Stoehr, A.M. 2002. Immune defense and host life history. *Am. Nat.* **160**: S9–S22. doi:10.1086/342131.
- Zuk, M., Bryant, M., Kolluru, G., and Mirovitch, V. 1996. Trade-offs in parasitology, evolution and behavior. *Parasitol. Today*, **12**: 46–47. doi:10.1016/0169-4758(96)80650-8. PMID:15275250.