Epidemiology of a Daphnia-Multiparasite System and Its Implications for the Red Queen

Stuart K. J. Auld1*, Spencer R. Hall2, Meghan A. Duffy1

1 School of Biology, Georgia Institute of Technology, Atlanta, Georgia, United States of America, 2 Department of Biology, Indiana University, Bloomington, Indiana, United States of America

Abstract

The Red Queen hypothesis can explain the maintenance of host and parasite diversity. However, the Red Queen requires genetic specificity for infection risk (i.e., that infection depends on the exact combination of host and parasite genotypes) and strongly virulent effects of infection on host fitness. A European crustacean (Daphnia magna) – bacterium (Pasteuria ramosa) system typifies such specificity and high virulence. We studied the North American host Daphnia dentifera and its natural parasite Pasteuria ramosa, and also found strong genetic specificity for infection success and high virulence. These results suggest that Pasteuria could promote Red Queen dynamics with D. dentifera populations as well. However, the Red Queen might be undermined in this system by selection from a more common yeast parasite (Metschnikowia bicuspidata). Resistance to the yeast did not correlate with resistance to Pasteuria among host genotypes, suggesting that selection by Metschnikowia should proceed relatively independently of selection by Pasteuria.

Introduction

Genetic specificity between hosts and their parasites shapes the ecology and evolution of infectious disease [1,2]. Specificity occurs in numerous host-parasite systems, and arises when the exact pairing of host and parasite genotypes determines the success of infection (e.g. [3,4,5,6,7]). Genetic specificity has the potential to influence disease phenomena in at least two ways. First, it decreases the likelihood of invasion of parasites into genetically diverse host populations (i.e., the parasite will have a lower reproductive ratio $R_0$ when all else is equal, [8]) because much of the host population resists infection. Indeed, genetically diverse populations often experience smaller disease epidemics [9,10,11,12,13]. Second, specificity means that each parasite genotype only selects against a subset of host genotypes (and vice-versa). Therefore, specificity can drive Red Queen coevolutionary dynamics, where the frequencies of host and parasite genotypes cycle through time [14,15]. Such coevolutionary cycling can maintain genetic diversity in both host and parasite populations [15,16].

The European freshwater crustacean Daphnia magna and its sterilizing bacterial parasite Pasteuria ramosa exemplify genetic specificity [5,7]. Here, specificity stems from a mechanism at the point of entry of Pasteuria – the physical attachment of spores to the host oesophageal wall [17], not from a systemic immune response [18,19]. If infection is successful, Pasteuria inflicts high virulence on infected hosts through sterilization [20,21]. Further, Pasteuria epidemics can become large [22,23,24], promoting rapid evolution in host populations [23]. In theory, such a combination of genetic specificity, high parasite virulence, and large epidemics promote Red Queen oscillatory dynamics [25,26,27]. As predicted by this theory, Red Queen dynamics emerge in the D. magna-Pasteuria system [20].

These results suggest that Pasteuria could maintain genetic diversity in other susceptible host species. Therefore, Pasteuria could act as a general catalyst of Red Queen-driven coevolution in freshwater systems. To test this possibility, we looked for genetic specificity for infection with Pasteuria in a natural North American host, Daphnia dentifera, using an established experimental design [7]. We also tested for evidence of high virulence in infected hosts, with particular focus on fecundity (since Pasteuria castrates its European D. magna hosts: [20]). If Pasteuria’s specificity and virulence effects are general, the European Daphnia – Pasteuria story may apply more broadly.

However, Red Queen dynamics could be disrupted by the presence of another, more dominant parasite species. For example, directional selection from a non-specific parasite may shape evolution in the host population more than negative frequency-dependent selection from a less prevalent parasite that exhibits genetic specificity with the host. This consideration is especially important in natural systems where most hosts are susceptible to multiple parasite species [29,30]. In D. dentifera populations, epidemics of the yeast Metschnikowia bicuspidata are usually larger and more frequent than Pasteuria epidemics [31,32]. Metschnikowia does not, however, exhibit genetic specificity with D. dentifera [33]; instead, variation in infection risk stems mainly from variation in host exposure to the parasite [34]. Since the infection genetics differ between the two parasites, a host that resists Pasteuria may not necessarily resist Metschnikowia. Such parasite-specific resistance (sensu [35]) may disrupt or prevent Red Queen
Received 100 levels among parasite species (based on pilot data). Sham controls Metschnikowia fecundity and survivorship. Although not previously studied in Pasteuria, infection also critically depends on attachment of cells to the osphagus of the host; this step depends strongly on genotype in the Daphnia magna system [17]. In both species, spore release follows death of the host (i.e., both are obligate killers: [39,40]), however, the two parasites vary in terms of their effects on fecundity and survivorship. Although not previously studied in Pasteuria, Pasteuria completely sterilizes D. magna [20]. In contrast, infection of both D. magna and D. dentifera by the yeast causes a smaller reduction in fecundity but sharply reduces survival of hosts [40,41,42]. In D. magna, Metschnikowia has a larger impact on host survival than does Pasteuria [39].

Experimental design

The experiments used host and parasite cultures and previously established laboratory protocols. The six clonal genotypes of Daphnia (named H1, H4, H9, H37 and H119) and five Pasteuria isolates (named A, B, C, D, and G) all originated from Midland Lake, Greene County, Indiana, USA. The five Pasteuria isolates were taken from five different infected hosts in 2010 (one isolate per host individual) and were propagated by feeding homogenized, infected hosts to a single host genotype (H119). These isolates likely consist of a mix of Pasteuria clones and represent ecologically relevant sub-populations of Pasteuria (the same as [7]). The Metschnikowia isolate originated from multiple infected hosts collected from Baker Lake, Barry County, Michigan, USA in 2003 and was propagated in a similar manner (on a single host genotype, the “Standard” genotype, also collected in Michigan). We used only this lab-based culture because Metschnikowia isolated from different lakes and in different years show no genetic variation in infectivity or virulence on D. dentifera [33]. No specific permits were required for the collection of D. dentifera (which is not a protected or endangered species), but permission was obtained for access to lakes.

The experimental design involved a factorial manipulation of these hosts and parasites. We crossed the six host genotypes with seven parasite treatments (five Pasteuria isolates, one Metschnikowia isolate, and a sham-exposure control), replicated 15 times, for a total of 630 experimental units. Four of the Pasteuria replicates were lost due to accidental death after treatment exposure. Maternal lines consisted of individual Daphnia kept under favourable conditions (20°C and 16:8 hour light/dark) in 40 mL of medium (50% Artificial Daphnia medium [43] and 50% filtered lake water); they received ample food (1×10⁶ cells of the alga Ankistrodesmus falcatus daily). Experimental animals consisted of second clutch offspring of third-generation maternal lines and were also kept in 40 mL of medium and fed ample food. In the parasite exposure treatments, each neonate (<24 hour old) received a high dose of spores (Pasteuria: 2000 spores/mL; Metschnikowia: 500 spores/mL) produced by gently crushing and diluting infected hosts. These doses aimed to yield similar infection levels among parasite species (based on pilot data). Sham controls received 100 μL of a Daphnia solution (50 uninfected hosts ground in 10 mL nanopure water). Replicates were fed lower food (0.5×10⁶ Ankistrodesmus algal cells) during exposure to increase spore uptake by hosts [38]. Treatment exposure lasted 5 days. After the exposure, each host was changed into fresh medium and given ample food (1×10⁶ Ankistrodesmus algal cells) again. Hosts were checked daily for reproduction and mortality for 25 days. Medium was refreshed every 2-3 days.

Data analysis

All analyses were performed using the statistical package R (http://www.r-project.org). We analysed infection risk (that is, the proportion of infected hosts) by testing the fixed effects of host genotype and parasite species using data from only parasite-exposed hosts (not sham-exposed hosts). We tested for genetic specificity of Pasteuria infection using the fixed effects of host genotype and parasite isolate. Both Generalized Linear Models (GLM) were estimated with a binomial error distribution, and significance of each treatment was evaluated with deviance tests (i.e., a comparison of full model versus the reduced model).

However, in previous Daphnia-parasite studies, genotype is fitted as a fixed effect, a random effect or both (e.g. [33,44,45,46]). Therefore, we also fitted GLMs with host and parasite genotype identity as random effects. In all cases, we obtained the same qualitative results. We also tested for a relationship between risk of infection from Metschnikowia and Pasteuria (averaged over the five isolates) for each host genotype using a Spearman rank correlation.

We examined the fitness consequences of infection by the two parasites using three metrics: production of host offspring (fecundity), host survival, and the instantaneous rate of host population growth, r (a composite measure of host fitness calculated using the Euler-Lotka equation). Fecundity was analysed by testing the fixed effects of infection status (infected or not), host genotype, and parasite species. Both host survival and r were analysed by testing the fixed effects of host infection status (infected or not) and parasite species. All of these tests used data from parasite-exposed hosts only; for r, the means of data from each infection category and parasite species for each host genotype were used.

Metschnikowia’s capacity to disrupt Red Queen dynamics between D. dentifera and Pasteuria should be reduced if the host pays an activation cost of resistance to Metschnikowia (i.e., if hosts that successfully resist Metschnikowia have a lower fitness than hosts that were not exposed to the parasite.) We looked for evidence of an activation cost of resistance to Metschnikowia by comparing host fecundity, survival, and r in controls (sham-exposures) with hosts that were exposed to parasites but did not suffer infection (sensu [47,49]). A Cox’s proportional hazards analysis was used to test for activation costs in terms of survival, and Welch’s two-sample t-tests were used to test for activation costs in terms of fecundity and r.

We found relatively low overall levels of infection by Pasteuria, as well as strong genotype specificity governing infections. This, combined with the likelihood that our parasite isolates contain mixtures of Pasteuria genotypes [3,48], means we cannot be sure that individuals in the Pasteuria exposures who did not become infected where exposed to a parasite spore of an infectious genotype. Thus, we cannot test for activation costs of resistance to Pasteuria. In any case, activation costs of resistance to Pasteuria are unlikely to be evolutionarily important because Pasteuria is so virulent. Since it castrates its hosts, the cost of Pasteuria infection is so massive that any cost of resistance would have to be exceptionally high to limit Red Queen dynamics.

Fecundity across treatments was examined using a GLM fit with a quasipoisson error distribution (used due to over-dispersion of the fecundity data), and survival analyses used Cox’s proportional
hazards. Measures of $r$ were squared to normalize their distribution prior to analysis with an ANOVA (with type III SS).

**Results**

Genetic specificity for infection risk

Genetic specificity for infection risk arose at both a within-species level (for the bacterium *Pasteuria*) and between parasite species. Infection risk from *Pasteuria* depended on the specific combination of host and parasite genotypes (Figure 1), as evidenced by a host genotype *x* *Pasteuria* isolate interaction (Table 1). This interaction remained significant when we treated both host genotype and parasite isolate as random effects ($\chi^2 = 15.39$, df = 3, $p<0.01$). As expected based on prior studies, host genotype strongly influenced infection risk from *Metschnikowia* (Figure 2; Table 1). Infection risk also depended on an interaction between host genotype and parasite species (Table 1). This interaction remained significant when host genotype was coded as a random effect ($\chi^2 = 22.81$, df = 2, $p<0.0001$). There was, however, no correlation between a genotype’s risk of infection by *Metschnikowia* and its overall risk of infection by *Pasteuria* ($r = -0.38$, $p = 0.46$; Figure 2).

Parasite virulence

Infection with either parasite substantially reduced fecundity in infected hosts (Figure 3A). However, the extent of this virulence on host fecundity differed between the parasites (i.e., there was a significant infection status *x* parasite species interaction; Table 2): *Pasteuria*-infected hosts suffered a greater reduction in fecundity than those infected with *Metschnikowia* (Figure 3A). The impact of infection on fecundity did not vary across host genotypes (that is, there was no infection status-by-host genotype interaction; Table 2). Host survival also strongly depended on infection status and parasite species: *Metschnikowia*-infected hosts died much earlier than either *Pasteuria*-infected hosts or uninfected hosts (Table 2; Figure 3B). Finally, the instantaneous rate of population growth ($r$; our composite measure of fitness) depended on both infection status and parasite species: infection with either parasite reduced $r$ more than *Metschnikowia* infections. Thus, *Pasteuria* was more virulent (Table 2; Figure 3C).

**Table 1. Summary statistics of models testing the effects of host genotype, parasite species and *Pasteuria* isolate on infection risk.**

| Source                                      | DF | L-R $\chi^2$ | $p$  |
|---------------------------------------------|----|--------------|------|
| Model 1: All parasite-exposed hosts         |    |              |      |
| Host genotype                               | 5  | 61.65        | <0.0001 |
| Parasite species                            | 1  | 0.13         | 0.72 |
| Host genotype *x* Parasite species          | 5  | 32.52        | <0.0001 |
| Model 2: *Pasteuria* only (5 isolates)      |    |              |      |
| Host genotype                               | 5  | 92.97        | <0.0001 |
| *Pasteuria* isolate                         | 4  | 35.06        | <0.0001 |
| Host genotype *x* *Pasteuria* isolate      | 20 | 42.47        | <0.01 |
| Model 3: *Metschnikowia* only (1 isolate)   |    |              |      |
| Host genotype                               | 5  | 13.22        | <0.05 |

DF: degrees of freedom; L-R $\chi^2$: likelihood-ratio $\chi^2$ statistic; $p$: p-value of test. Bolded values are significant at alpha = 0.05 level. doi:10.1371/journal.pone.0039564.t001

Costs of resistance to *Metschnikowia*

Host fecundity did not differ between hosts that were exposed to *Metschnikowia* but not infected and those in our control treatment (control, $n = 90$; *Metschnikowia*-exposed, $n = 69$; Welch’s two-sample $t = 0.45$, df = 110.74, $p = 0.65$; Figure 3A); neither host survival (Cox’s proportional hazards: $\chi^2 = 0.10$, $p = 0.73$; Figure 3B) nor the instantaneous rate of host population growth, $r$ (Welch’s two-sample $t = 0.14$, df = 7.31, $p = 0.89$; Figure 3C) differed between these two groups (that is, *Metschnikowia*-exposed and control). Thus, there was no evidence for a fitness cost of mobilising resistance mechanisms against *Metschnikowia* (Figure 3).

Figure 1. Infection risk for six host genotypes exposed to five isolates of *Pasteuria ramosa*. The matrix shows the proportion of hosts infected for each combination of host genotype (rows) and parasite isolate (columns). Cells show infectivity of the five different *Pasteuria* isolates on each of the six host genotypes. There are five shading categories: 0% infection (white), 1–20%, 21–40%, 41–60%, and 61–100% (black). See Table 1 for statistical details. doi:10.1371/journal.pone.0039564.g001

Figure 2. Spearman rank correlation between *Pasteuria* and *Metschnikowia* infectivity, $r_s = -0.38$, $p = 0.46$. Each data point is the mean for each host genotype. doi:10.1371/journal.pone.0039564.g002
Red Queen dynamics require two essential ingredients. The first is genetic specificity, where infection depends on the precise combination of host and parasite genotypes [15]; the second is high virulence [25,26], where infection by parasites causes dramatic loss of host fitness. A canonical example of genetic specificity occurs in the European Daphnia magna-Pasteuria host-parasite system [5,7]. We found Pasteuria produces a similar pattern of specificity in a North American host, Daphnia dentifera: infection risk depended on both host genotype and parasite isolate (Figure 1). Also, just as in D. magna, Pasteuria castrates D. dentifera, thus greatly depressing fecundity (but not survival: Figure 3; [40,50]). As a result, fitness is much reduced in Pasteuria-infected D. dentifera (Figure 3). Therefore, just as in the D. magna system, Pasteuria in this North American system might also maintain host genetic diversity, become adapted to locally common host genotypes [51,52], and promote long-term coevolutionary Red Queen dynamics [28].

In addition to Pasteuria, D. dentifera populations often encounter Metschnikowia, a very different parasite. Infection of D. dentifera by Metschnikowia does not depend on the specific genotype to which it is exposed [33]; instead, genetic variation for host susceptibility stems from host variation in risk of exposure to the parasite [34]. This may explain the absence of activation costs of resistance to Metschnikowia: hosts that resisted the parasite may have prevented it from passing the gut wall and stimulating a potentially costly immune response (sensu [18]).

Here, we found that virulence caused by Metschnikowia arose mainly through reduced host survival in D. dentifera (Figure 3B; Table 2). Data were pooled across host genotypes and parasite isolates. Host r was analysed using the means for each host genotype. *p<0.05, **p<0.01, ***p<0.001.

doi:10.1371/journal.pone.0039564.t002

Table 2. Summary analysis of fitness components of hosts: fecundity, survival, and instantaneous rate of population growth, r.

| Host fitness measures | Host fecundity | Host survival | Host r |
|-----------------------|----------------|---------------|--------|
| All parasite-exposed hosts | F1,535 = 298.69*** | x2 = 4.94* | F1,59 = 12.80*** |
| Infection status (Inf) | F5,535 = 2.30* | - | - |
| Host genotype (Host geno) | F5,535 = 9.35** | - | F1,59 = 6.78 |
| Parasite species (Para sp.) | Inf x Host geno | F1,535 = 0.16 | x2 = 9.35** | F1,59 = 9.58 |
| Host genotype x Para sp. | Inf x Para sp. | F1,535 = 12.92*** | x2 = 9.35** | F1,59 = 13.00*** |
| Pasteuria only (5 isolates) | Host genotype x Para sp. | F1,535 = 0.91 | - | - |
| Pasteuria isolate | F1,535 = 0.06 | - | - |
| Metschnikowia only (1 isolate) | Pasteuria isolate | - | - | F5,47 = 0.95 |

Figure 3. Measures of host fitness in different treatments according to infection status. A: production of host offspring (fecundity) over 25 days; B: survival of hosts; and C: instantaneous rate of population growth of hosts (r), a composite measure of host fitness.
similar to Metschnikowia infection in D. magna hosts. While Metschnikowia killed hosts relatively quickly, it reduced host fecundity much less than Pasteuria did (Figure 3A). As a result, Metschnikowia reduced per capita host fitness (r) to a lesser extent than Pasteuria (Figure 3C). Nevertheless, despite the lack of genetic specificity and lower virulence, Metschnikowia can still drive evolutionary change of hosts during epidemics. Indeed, large Metschnikowia epidemics can substantially reduce host population densities [31] and impose both directional and disruptive selection on host populations [33,54].

Due to differences in specificity and virulence, these two parasites may exert different ecological impacts on host populations. Theory suggests that specificity can reduce invasion success of parasites; i.e., they will have a lower reproductive ratio R0 (especially when host populations are genetically diverse: [8]). Indeed genetically diverse host populations of Daphnia magna have smaller epidemics of Pasteuria [9,10]. Specificity may thus (partially) explain why Pasteuria epidemics occur more rarely and remain much smaller than epidemics of Metschnikowia in natural populations of D. dentifera [32]. However, even though they are relatively small, Pasteuria epidemics may still be ecologically important: theory predicts that, all else being equal, parasites that strongly reduce fecundity (rather than survival) should reduce host population densities, even during small epidemics [55].

Correlations between host resistance to Pasteuria and Metschnikowia could either amplify or dampen Red Queen oscillations. A negative correlation would indicate antagonistic pleiotropy, where resistance to Pasteuria would come at a cost to resistance to Metschnikowia [56]; this could bolster Red Queen dynamics. Conversely, a positive correlation would imply that hosts might use a general mechanism to resist both parasite species. In this positive correlation case, directional selection for generally resistant hosts could erode genetic variation for resistance in host populations and squish Red Queen dynamics [57] – provided that this general resistance is not traded-off against another parasite-independent host trait [58]. This study found no correlation between host resistance to the two parasites (Figure 2), probably because of very different underlying resistance mechanisms involved [17,34]. We acknowledge, however, that a more powerful design using more host genotypes could more comprehensively exclude the possibility of correlations between resistance to these two parasites. The lack of a correlation between resistance to Metschnikowia and Pasteuria suggests evolution of D. dentifera in response to the two parasites should proceed relatively independently. Thus, we do not expect Metschnikowia epidemics to amplify or dampen Red Queen dynamics between D. dentifera and Pasteuria.

In conclusion, Pasteuria could promote genetic diversity in multiple host species through host-parasite coevolution. Pasteuria exhibits strong genetic specificity and high virulence with its North American host, D. dentifera, just as it does with its European host, D. magna. Can Pasteuria then promote host diversity in North American lakes? We cannot yet fully answer that question with these or other data, but the D. dentifera-Pasteuria system in isolation certainly contains several of the essential ingredients of the Red Queen. Still, it seems likely that the larger epidemics of the more common Metschnikowia yeast may mean that parasite-mediated selection in D. dentifera is driven primarily by Metschnikowia, making Red Queen dynamics relatively rare. This possibility further highlights the need to consider other co-occurring parasites when exploring host-parasite coevolution.

Acknowledgments

K. Boatman, M. Shocket, and Z. Brown helped collected hosts and parasites. We thank Anna Moynihan and two anonymous reviewers for helpful comments on a previous version of the manuscript.

Author Contributions

Conceived and designed the experiments: SKJRA MAD. Performed the experiments: SKJRA. Analyzed the data: SKJRA MAD. Contributed reagents/materials/analysis tools: SRH. Wrote the paper: SKJRA SRH MAD.

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