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Parasite-driven replacement of a sexual by a closely related asexual taxon in nature

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Abstract. Asexual species are thought to suffer more from coevolving parasites than related sexuals. Yet a variety of studies do not find the patterns predicted by theory. Here, to shine light on this conundrum, we investigate one such case of an asexual advantage in the presence of parasites. We follow the frequency dynamics of sexual and asexual *Daphnia pulex* in a natural pond that was initially dominated by sexuals. Coinciding with an epidemic of a microsporidian parasite infecting both sexuals and asexuals, the pond was rapidly taken over by the initially rare asexuals. With experiments comparing multiple sexual and asexual clones from across the local metapopulation, we confirm that asexuals are less susceptible and also suffer less from the parasite once infected. These results are consistent with the parasite-driven, ecological replacement of dominant sexuals by closely related, but more resistant asexuals, ultimately leading to the extinction of the formerly superior sexual competitor. Our study is one of the clearest examples from nature, backed up by experimental verification, showing a parasite-mediated reversal of competition dynamics. The experiments show that, across the metapopulation, asexuals have an advantage in the presence of parasites. In this metapopulation, asexuals are relatively rare, likely due to their recent invasion. While we cannot rule out other reasons for the observed patterns, the results are consistent with a temporary parasite-mediated advantage of asexuals due to the fact that they are rare, which is an underappreciated aspect of the Red Queen Hypothesis.

Key words: *asexual;* Daphnia*; ecological replacement; host–parasite interaction; invasion biology; microsporidia; reproductive mode.*

Introduction

Closely related species are often infected by the same parasites, but differ in their susceptibility to and fitness reduction from parasite-inflicted harm (e.g., Thomas et al. 1995, Stirnadel and Ebert 1997, Friesen et al 2018). Such asymmetries can render less resistant species poorer competitors, and more resistant species may even competitively exclude less resistant ones (Thomas et al. 2005, Hatcher et al. 2006). Parasites may thus have strong effects on community structure and the distribution of host species, especially if the competitive dominance relationships among hosts are inversed in the presence of parasites (Thomas et al. 2005, Hatcher et al. 2006). Several high-profile empirical examples of parasite-mediated competition are known (Park 1948, Schall 1992, Thomas et al. 1995, Tompkins et al. 2003). However, clear examples from natural populations of parasite-mediated reversals of competitive dominance relationship leading to the ecological replacement of a formerly dominant species by a more resistant competitor are virtually unknown.

Two circumstances under which parasite-mediated competitive effects may play a particularly important role are in the context of biological invasions and in competitive interactions between sexuals and closely related asexual species. Theory on host–parasite interactions predicts that asexual species should suffer more, on average, from parasites compared to their sexual relatives. This is due to their lower genetic diversity and reduced ability to generate new allelic combinations, which are predicted to lead to a higher average parasite load (Jaenike 1978, Lloyd 1980, Hamilton 1980, Hamilton et al. 1990). This is thought to prevent asexuals from replacing sexuals within populations, because asexuals are thought to become increasingly parasitized as they become more common. The prediction that asexuals should be overparasitized has been investigated

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empirically in many systems, with some studies finding evidence in support of the prediction (Lively and Jokela 2002, Kumpulainenet et al. 2004, Vergara et al. 2014) and others not (Hanley et al. 1995, Ben-Ami and Heller 2005, Elzinga et al. 2012; see also review by Neiman et al 2018).

Such variable results are not necessarily inconsistent with theory: asexuals are only predicted to be over-parasitized on average, not in every single case. Specifically, when asexuals are rare, parasites may be adapted to the more common sexuals, and asexuals may therefore suffer less from parasites than sexuals, at least temporarily (Lively et al. 2004, Lively 2009, Gibson et al. 2018). One such case where asexuals might suffer less from parasites than sexuals occurs in the context of biological invasions. Asexuals are thought to be particularly prone to become invasive (Vandel 1928, Baker 1955, Peck et al. 1998, Haag and Ebert 2004), and indeed, there are empirical data showing that asexuals are overrepresented among invasive species (Ellstrand and Schierenbeck 2000, Sakai et al. 2001). Furthermore, especially during the early phases of invasions, invasive species may suffer less from parasites than native competitors because parasites tend to be more adapted to native species or genotypes, with whom they share their recent coevolutionary history (Torchin et al. 2003).

Here, we document the rapid replacement of a sexual by a closely related asexual taxon in nature and show experimentally that this replacement was very likely driven by parasites. We discuss the results with respect to sexual-asexual predictions, as well as with respect to invasion biology (the asexual taxon is invasive). Specifically, we studied cyclical and obligate parthenogenetic lineages of the freshwater cladoceran *Daphnia pulex* that occur in small rock pools to form dynamic metapopulations across the Skerry archipelago of Southern Finland (Pajunen 1986, Pajunen and Pajunen 2003, Lehto and Haag 2010). In winter, the ponds freeze to the bottom and only diapause stages survive. In our study area, about 5–10% of the rock pools harbor *D. pulex*. Cyclical and obligate parthenogenetic *D. pulex* often inhabit different ponds, in part due to chance events caused by the dynamics of extinction and recolonization and in part due to different niche preferences with respect to water chemistry, in particular pH and calcium abundance. In ponds with intermediate water chemistry, cyclical and obligate parthenogenetic populations coexist (Lehto and Haag 2010).

Obligate asexual lineages belong to the North American clade of *D. pulex* and have a history of introgression and contagious asexuality with cyclical parthenogens of the same clade, as well as the closely related *Daphnia pulicaria* (Innes and Hebert 1988, Tucker et al. 2013, Xu et al. 2015). It is unknown exactly when the asexuals were introduced to Europe, but it was probably near the beginning of the 20th century in the ballast water of transport ships returning from the Laurentian Great Lakes, a transport route common to many aquatic invasive species (Carlton 1985, Williams et al. 1988). The cyclical parthenogenetic *D. pulex* in our study system belong to the European clade, which, however, may represent a different species (Mergeay et al. 2008, Marková et al. 2013). In the absence of a formal description of the two taxa as different species, we here still use the species name *D. pulex* for both, but refer to them as closely related taxa. Obligate asexuals (hereafter "asexuals") produce both live-born offspring and diapausing stages asexually, whereas cyclical parthenogens (hereafter "sexuals") have several asexual generations (production of live-born offspring) and typically one sexual generation (production of diapause stages) per year (Innes and Hebert 1988, Paland et al. 2005, Lynch et al. 2008, Heier and Dudycha 2009). Asexuals are genetically highly uniform, whereas sexuals show moderate levels of genetic variation, typical of other cyclical parthenogenetic *Daphnia* species (Ward et al. 1994, Haag et al. 2005, Walser and Haag 2012).

We followed the frequencies of sexuals and asexuals in one mixed natural pond over 14 yr (1999–2013). In 2006, a strong epidemic caused by a highly virulent parasite, *Gurleya vavrai*, coincided with the total replacement of formerly dominating sexuals by asexuals. The parasite, *G. vavrai*, is an endemic microsporidian pathogen in Europe (Green 1974, Refardt et al. 2002), with no record of the parasite occurring in North America. Infections are localized in the epidermis and cause a progressive whitening of the body as the infection spreads, with high virulence (Friedrich et al. 1996, Stirnadel and Ebert 1997, Little and Ebert 1999). Using a series of field and laboratory experiments, we assessed the relative competitiveness of sexuals and asexuals in the presence and absence of the parasite, the susceptibility of sexual and asexuals hosts, as well as life history traits of infected and uninfected individuals. These experiments were designed to assess if in the entire metapopulation, asexuals have an advantage in the presence of parasites, and if the reversal in relative abundance observed in the natural pond was likely caused by parasites.

Methods

Monitoring of the natural pond

One pond, SK-39 (59°49'55.7" N 23°15'17.6" E, pond surface 1.6 m^2, depth 0.3 m) on the island of Skallothomen, contained both sexuals and asexuals in an initial survey in 1999. In 2000 and again in 2008, no animals were observed in the pond during routine visits, possibly because appropriate conditions for the hatching of diapause stages did not occur in those years. In all other years, namely 1999 to 2013, *D. pulex* were present, with estimated sizes of the active (i.e., non-diapausing) population of >10,000 individuals at any given time. We monitored the frequencies of sexuals and asexuals in a total of 26 samples taken between 1999 and 2013 (total N = 1,848 individuals, for exact sampling dates and

sample sizes per date, see data deposited on the Dryad digital repository). The breeding type (sexual, asexual) of each individual in these samples was assessed using cellulose-acetate electrophoresis (Hebert and Beaton 1993) at the PGI locus (phosphoglucose isomerase, EC 5.3.1.9.), for which the sexuals and asexuals have consistently different genotypes (Lehto and Haag 2010). On 14 July 2006, we noted a strong *G. vavrai* infection in our samples. Even though previous samples were not systematically checked for this parasite, a previous epidemic would not have gone unnoticed. We subsequently took additional samples to estimate the parasite prevalence in sexuals and asexuals (20, 25, and 30 July, 29 September). Prevalence was assessed by visually inspecting the carapace color with light from the top and against a dark background. Checking for a white carapace detects only late-stage infections, while early-stage infections are pooled with the uninfected animals. Thus, prevalence estimates are most likely underestimates. From 2007 to 2013, we recorded only presence/absence of infections.

Outdoor experiment: Experimental design

We carried out a multi-generation competition experiment in outdoor containers (40-L buckets) to test for the relative competitiveness of sexuals and asexuals in presence and absence of the parasite. The experiment used sexuals and asexuals sampled across the metapopulation (specifically from 20 geographically distinct ponds, of which 10 "sexual ponds" were inhabited only by sexuals and 10 "asexual ponds" were only inhabited by asexuals, Appendix S1: Table S1). In each competition trial, animals from one of the sexual ponds were grown together (i.e., in the same bucket) with animals from one asexual pond. For this, the 20 ponds were randomly assigned to 10 pairs, each consisting of one sexual and one asexual (Appendix S1: Table S1). Each pair was used for two competition cultures, one was grown in presence, the other in absence of the parasite. This paired design was chosen to ensure statistical independence of replicates between pairs (animals in different pairs originated from different ponds). The fact that replicates of the same pair used animals originating from the same ponds was taken into account in the statistical analysis by including "pair identity" as a random factor. Ensuring statistical independence of pairs with respect to origin of samples was important because the experiment was designed to test the more general question of whether an average asexual in this metapopulation suffers less from infection (and/or is less susceptible) than an average sexual. It indirectly assesses also whether the changes in relative abundance of sexuals and asexuals in SK-39 were likely driven by the parasite (the more direct test using animals only from SK-39 was technically impossible because sexuals were extinct in that pond at the time the experiments were carried out).

Outdoor experiment: Origin and handling of hosts and parasites

Samples from natural populations were obtained in May 2010 and used to establish outdoor cultures (one separate culture per population) in buckets, which were left outdoors under ambient conditions on the island of Furuskär (59°49'58" N 23°15'49" E). Before introducing the *Daphnia*, each bucket was filled with 40 L of 0.02-mm double-filtered water from a nearby pond, not colonized by *Daphnia*. Each bucket was stocked with ~100 randomly chosen individuals (which should relatively well represent the genetic diversity in the pond of origin), and the cultures were then left for two months to reproduce and increase in numbers and to acclimate to experimental conditions. This pre-experimental period was important to minimize potentially confounding effects of the environment of origin. The only exception to the procedure was a bucket containing asexuals from SK-39, which had already been started using a single, uninfected individual in 2007. The breeding type (sexual, asexual) of each culture was confirmed using the PGI locus as described above. In July 2010, these cultures were harvested and used as the experimental animals for the outdoor experiment. Cultures were inspected to ensure the absence of any infected individuals at the start of the experiment.

The parasites used in the experiment were obtained from four additional cultures of *D. pulex*, which had been maintained in buckets on the island of Furuskär from 2007 to 2010 with *G. vavrai* infections. Two of these cultures contained sexuals, the two others asexuals, (Appendix S1: Table S1). Infected individuals from all four cultures were pooled to produce the spore cocktails used for infection in this experiment.

Outdoor experiment: Experimental set-up

For the actual experiment, we prepared 20 fresh 40-L buckets with filtered pond water (filtered from a representative pond, without *Daphnia* populations to avoid bias). Into each of these, we transferred 100 sexuals (from one pre-experimental culture) and 100 asexuals (from another pre-experimental culture), according to the random pairings described above. Each pair was replicated twice, one replicate per pair was exposed to *G. vavrai* spores and the second served as the control culture without parasite exposure. Infected *D. pulex* were ground up and distributed equally across the 10 infection replicates (approximately 8.5×10^8 spores per bucket). In the control treatment, a comparable amount of ground-up, uninfected *D. pulex* were added as a placebo.

Outdoor experiment: Recorded parameters

The cultures were left in the field, and samples were obtained from each culture at three time points: August

2010, September 2010, and October 2010. At each sampling event, 50 individuals were removed per culture and checked visually for infection. Subsequently, the same 50 individuals were typed as sexual or asexual using the PGI locus. In this way, the prevalence of infection in sexuals versus asexuals could be monitored over the course of the experiment. Note that we designed a paired competition experiment to measure relative frequency changes between treatments. The overall frequency changes are difficult to interpret because they are influenced by many factors, such as water chemistry (Lehto and Haag 2010). Note also that these estimates do not include potential differences in the production of diapause stages.

Laboratory experiment: Origin and handling of hosts and parasites

In June 2013, *D. pulex* were collected from various ponds in the Tvärminne area. Both sexuals and asexuals were collected, as well as *G. vavrai*-infected individuals of both taxa (Appendix S1: Table S1). All individuals were transported live to the laboratory in Switzerland for immediate use. In the laboratory, one clonal line (lines started by single females and maintained exclusively by clonal reproduction in both the sexuals and asexuals) was established from each of the pond samples. Each line was propagated in a 400-mL glass jar filled with a water medium designed for *Daphnia* culturing (ADaM; Klüttgen et al. 1994) and fed with unicellular green algae (*Scenedesmus obliquitus*) ad libitum under a summer photoperiod of 16 h light: 8 h dark and a temperature of 20°C. The lines were maintained in this way for two weeks, and their breeding type was confirmed using the PGI locus.

Two separate *G. vavrai* cultures were established in the laboratory: one obtained from and grown on sexuals and a second obtained from and grown on asexuals. These parasite types are referred to as sexual and asexual *G. vavrai* isolates, respectively, referring to their host type. In both cases, the *D. pulex* hosts on which the spores were grown were those on which the parasite was collected, which were different clones from those used in the experiments (Appendix S1: Table S1).

Laboratory experiment: Experimental procedures

For the infection experiment, we used eight clonal lines (four sexual lines and four asexual lines) with 30 replicate individuals per line and each line originating from a different pond (Appendix S1: Fig. S1, Table S1). We started by isolating 50 individuals from each of the clonal lines (each placed individually in a 50-mL falcon tube) to ensure we would have at least 30 individuals at the beginning of the experiment. The animals were fed daily with 2.5×10^6 cells of the algae *S. obliquitus* and were kept in a climate chamber with a photoperiod of 16 h light: 8 h dark and a temperature of 20°C. The ADaM culture medium was changed three times per week (Monday, Wednesday, and Friday). The animals were passed through three generations under these pre-experimental conditions in order to remove maternal effects. Day zero of the experiment was when the fourth-generation offspring (third-clutch offspring of the third-generation females) were isolated into new tubes.

For infection, two separate spore cocktails were prepared: one from ground-up sexual hosts infected with *G. vavrai* and another from ground-up infected asexual hosts infected with *G. vavrai*. Infected hosts came from two separate ponds for both the sexual and asexual cultures (Appendix S1: Table S1). The total number of spores was equalized between the two treatments (spore numbers were estimated using a Neubauer improved counting chamber) and then distributed equally over the respective tubes. This resulted in approximately 60,000 *G. vavrai* spores being added to each tube in the parasite treatments. For the control treatment, a cocktail of ground-up uninfected *D. pulex* was added to the tubes.

Offspring from 30 mothers per clone (and eight clones: four sexual, four asexual) were used as the experimental animals, resulting in a cohort of 240 animals at the start of the experiment. Each clonal line then received three different treatments, with 10 replicates per treatment: (1) 60,000 spores of *G. vavrai* from asexual hosts, (2) 60,000 spores of *G. vavrai* from sexual hosts, (3) ground-up, uninfected *D. pulex* (control).

Laboratory experiment: Recorded parameters

Recorded parameters from the experiment were age at first reproduction, reproductive output (total number of offspring), and age at death, as well as infection status and number of spores at death. To determine the number of spores at death, each individual was homogenized in 0.3 mL of medium and the concentration of spores was determined using a Neubauer improved counting chamber.

Data analysis

All statistical analyses were performed using the software R (R Core Team 2013). For the outdoor competition experiment, we used a generalized linear-mixed model with a binomial error distribution to look at differences in the frequency of the sexuals vs. asexuals (R program lme4). For data analyses, each sexual individual was coded as 1, and each asexual individual as 0. We used treatment (control, parasite-exposed) and sampling time point as fixed factors, whereas replicate culture and pond pair (i.e., the pair of ponds, one inhabited by sexuals the other by asexuals, from which animals were used for a given competition culture) were treated as random factors, with replicate culture nested within treatment: breedingtype ~ treatment × time + (1|treatment/replicate) + (1| pair of ponds), family = binomial. We used a similar generalized linear-mixed model to investigate differences

in parasite prevalence (proportion of individuals infected, each individual being counted as either infected or uninfected) between the sexual and asexual cultures, but here we used the parasite-exposed treatment only: infection_status ~ breedingtype × time + (1|replicate), family = binomial. We verified model assumptions and absence of over-dispersion by calculating the sum of the squares of the Pearson residuals and comparing them with the residual degrees of freedom using a chi-squared test.

Data on age at death, reproductive output, and age at first reproduction from the laboratory experiment were evaluated using linear-mixed models in R, with the nlme package. Breeding type (asexual or asexual), and treatment (control, infected) were set as fixed factors and clone as a random factor: trait ~ breedingtype × treatment, random = ~(1|breedingtype/clone). Only those individuals that successfully became infected were used for comparison between treatments. We then used the same analysis exclusively on the infection treatment (and those individuals that actually became infected), but now used spore origin instead of treatment to test whether the different life history traits were affected by whether the *G. vavrai* spores were derived from sexual or asexual host clones. Using the same data, we also tested for differences in spore load at death using the same model as for the life history traits, as well as for differences in parasite prevalence (number of exposed individuals that became infected). For the latter, we used a generalized linear-mixed model, with infection success as the response variable (1, successfully infected; 0, infection failed) and a binomial error distribution. Breeding type and spore type (sexual or asexual host origin) were held as fixed factors, whereas clone was a random factor nested within breeding system: infection status ~ breedingtype × sporetype, random = ~1|breedingtype/clone, family = binomial. As above, we verified model assumption and absence of over-dispersion before running the analyses.

Results

Dynamics in the natural pond

There were strong seasonal dynamics in the relative frequencies of sexuals and asexuals (Fig. 1). Asexuals were more frequent early in the season, and sexuals dominated in late season. Yet, across years, the starting frequencies of sexuals increased (reaching 93% on 1 June 2006), as the pond became more and more dominated by sexuals. By the end of June 2006, the frequency of sexuals had increased to 98%. Then, on 14 July 2006, we detected many individuals (sexuals and asexuals not distinguished) with *Gurleya vavrai* infections, and at the same time a dramatic decrease in the frequency of sexuals to 41%. This decline continued over the course of the growing season down to 0% (9% on 20 July, 4% on 25 July, 2% on 30 July, and 0% on 28 September). In the following years (two samples in 2007, and one in each of 2010, 2012, and 2013), we found only asexuals (Fig. 1).

No quantitative measure of prevalence was recorded on 14 July 2006, but we noted many infected individuals. On 20 July, many dead, infected individuals were found in the pond, the breeding type of which could not be assessed. Among those living, we found 10 infected individuals in a sample of 54 (prevalence = 19%). Of the 10 infected individuals, 5 were sexuals and 5 were asexuals, whereas all of the uninfected individuals were asexuals (Fig. 1; Fisher's exact test, $P < 0.0001$). Prevalence then rapidly dropped to low levels (2% on 25 July, 4% on 30 July, and 7% on 28 September). The higher prevalence in sexuals was still significant on 25 July (infected: 3 sexuals, 5 asexuals; uninfected: no sexuals, 72 asexuals, $P = 0.0007$), but not anymore on 30 July, when all 13 infected individuals were asexuals, whereas 2 among 74 uninfected individuals were sexuals ($P > 0.5$). The latter result suggests that not all sexuals became infected and died during the epidemic or that a few individuals were able to clear the infections (though as pointed out above,

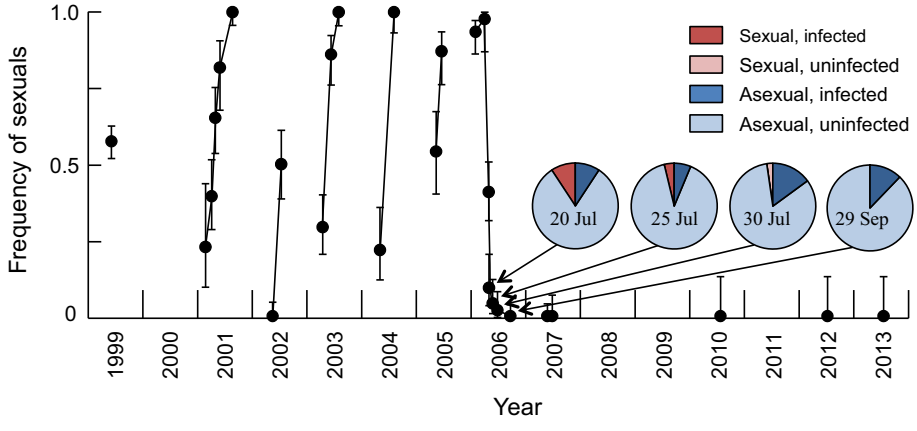

Fig. 1. Frequency of sexuals in pond SK-39. Samples taken in the same year are connected with a line. The error bars correspond to 95% confidence intervals according to the modified Wald method (Agresti and Coull 1998). The four pie charts represent data on prevalence whenever systematically recorded (red, sexuals; blue, asexuals; dark, infected; light, uninfected).

light infections might have gone unnoticed). From 2007 to 2013, when only asexuals were left in the pond, we recorded infected individuals in all samples, though the infections were never as clearly abundant as during the beginning of the epidemic.

Outdoor experiment

Asexuals performed relatively better in the competition experiment in the presence of the parasite *G. vavrai* than in their absence (Fig. 2). The infected cultures had higher frequencies of asexuals than the control cultures (September, 0.46 infected culture vs. 0.26 uninfected control; October, 0.46 infected culture vs. 0.28 uninfected control; $z = -3.04$, df = 2, $P = 0.002$). This was true at both sampling time points and thus sampling time was not a significant factor in the linear-mixed model ($z = 0.60$, df = *2,* $P = 0.551$). In addition, in the parasite treatment, a greater proportion of sexuals became infected, as opposed to asexuals (September, 0.34 sexual vs. 0.14 asexual; October, 0.39 sexual vs. 0.14 asexual; $z = 2.21$, df = 2, $P = 0.038$; Fig. 2). Again sampling time point was not significant ($z = 0.41$, df = 2, $P = 0.389$).

Laboratory experiment

In line with the results from the outdoor experiment, 76.3% of the sexual individuals exposed to *G. vavrai* spores became infected, whereas only 25.0% of the asexuals did so ($z = 4.96$, df = 2, $P < 0.001$; Fig. 3a). The comparison of the infected individuals (spore origins not distinguished) with the uninfected controls revealed clear negative fitness effects of infection: infected individuals died sooner ($t = -5.14$, df = 149, $P < 0.001$) and were less fecund ($t = -5.90$, df = 149, $P < 0.001$), while there was no significant effect for age at maturity ($t = 1.47$, df = 149, $P = 0.143$; Fig. 4). In no case was breeding system a significant factor on its own, suggesting that there was no clear main difference in life history traits between sexuals and asexuals under the experimental conditions (age at death, $t = 0.52$, df = 6, $P = 0.620$; age at maturity, $t = 0.04$, df = 6, $P = 0.972$; reproductive output, $t = 1.60$, df = 6, $P = 0.163$). However, there was a significant interaction between breeding type and infection for reproductive output ($t = -3.10$, df = 149, $P = 0.002$), suggesting that infection reduced the reproductive output of sexuals more strongly than that of asexuals, whereas asexuals had a somewhat lower reproductive output in the controls. The interaction was nonsignificant for age at death ($t = -1.02$, df = 149, $P = 0.307$) and for age at maturity ($t = -0.51$, df = 149, $P = 0.614$).

Looking only at the infected individuals, there was no significant difference in spore load at death between sexual and asexual *Daphnia* ($t = -0.60$, df = 71, $P = 0.553$; Fig. 3b). Furthermore, the spore origin did not appear to affect infection success or spore load, either overall or differently between sexuals and asexuals (nonsignificant main effects: infection success, $z = 1.03$, df = 2, $P = 0.304$; spore load at death, $t = 0.20$, df = 150, $P = 0.845$; and nonsignificant interactions with breeding type: infection success, $z = -1.28$, df = 2, $P = 0.200$; spore load at death, $t = 0.64$, df = 71, $P = 0.522$). However, spore origin affected the reproductive output. Spores obtained from asexual hosts reduced the reproductive output of asexuals more than spores obtained from sexual hosts, whereas no such effect was observed in the sexuals (breeding type × spore type interaction, $t = -2.85$, df = 71, $P = 0.006$). Finally, spore origin did not affect age at death, or age at maturity (breeding type × spore type interaction; age at death, $t = -1.51$, df = 71, $P = 0.135$; age at maturity, $t = -0.25$, df = 71, $P = 0.805$; Fig. 4).

Discussion

Using a metapopulation system, this study documents the replacement of an initially dominating sexual taxon by an initially rare asexual taxon in nature. This replacement did not occur gradually, as would be expected if the asexuals had an overall higher fitness. Rather, the replacement happened rapidly and was tightly linked with an epidemic caused by a virulent parasite, which

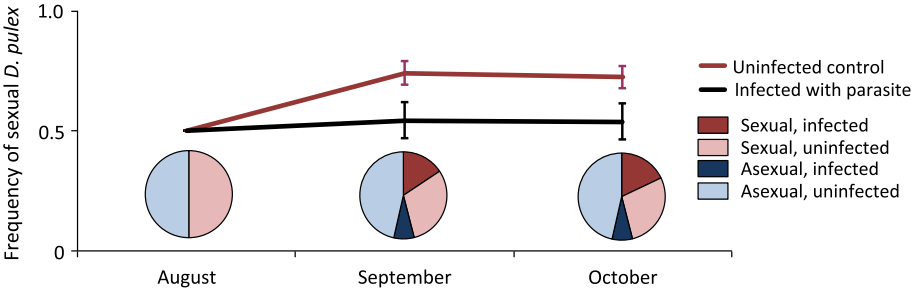

Fig. 2. Outcome of the competition experiment between asexual and sexual *Daphnia pulex* exposed to *Gurleya vavrai* in the summer of 2010. In August, the starting point of the experiment, there were an equal number of sexual and asexual individuals. Line graphs show the change in the frequency of sexuals and asexuals over the three-month experiment. Error bars represent standard errors of the means across 10 replicates. Pie charts show the proportion of the sexuals and asexuals infected with *G. vavrai* (red, sexuals; blue, asexuals; dark, infected; light, uninfected).

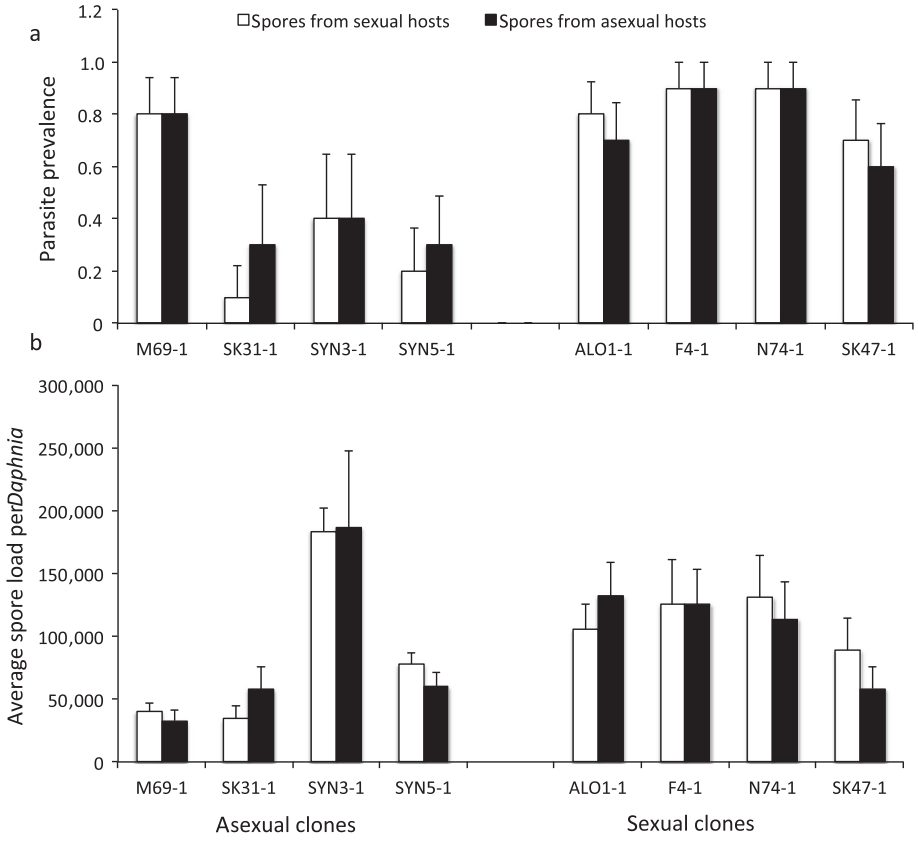

FIG. 3. (a) Parasite prevalence for the four asexual and four sexual clones used in the laboratory experiment, and (b) spore load at death. Error bars show the standard error of the mean values.

infected both sexuals and asexuals. Note that it is likely that sexuals were present in the sediment as diapause stages, even after 2006. However, they never made it back into the active population, at least not in any noticeable frequency, perhaps because the parasite kept being present in the pond in the asexual hosts. In either case, our field and laboratory experiments strongly support a causal role of the parasite in the replacement: asexuals were less susceptible to infection, suffered less from infection than sexuals, and their relative performance in a competition experiment was enhanced in the presence of parasites. Independent of the reproductive mode of the two competitors, this shows that parasites can strongly alter competitive interactions between closely related taxa. Parasites have been implicated in ecological replacements between closely related species several times before, including in the replacement of residents by invaders (Tompkins et al. 2003, Thomas et al. 2005, Hatcher et al. 2006). Though, to our knowledge, in none of these previous cases could the replacement be monitored so closely in nature and be supported by experimental evidence for a causal role of the parasite.

The results of our experiments show that, across the entire metapopulation, asexuals have an increased performance relative to sexuals in the presence of the parasite. In the laboratory experiment, the effects were strong: infection rates were higher and infection resulted in stronger negative fitness effects in sexuals than in asexuals. Nonetheless, the frequency changes in the outdoor competition experiment were much less dramatic than in SK-39. The potential reasons for the different frequency dynamics between the natural population and the outdoor experiment are difficult to evaluate. Yet, a hint may be obtained from the different observed infection rates. Indeed, in the natural population, virtually all sexuals were infected at the moment of the strong frequency changes. Also in the laboratory experiment, sexuals had high infection rates (~75%). In contrast, in the outdoor competition experiment, infection rates among sexuals were only between 30% and 40%. These difference in infection rates occurred despite the fact that outdoor cultures received a higher number of spores per volume and per initial host individual than the laboratory cultures. However, the outdoor cultures were carried out in deep buckets, and there is a possibility that spores got deposited on the bottom of the buckets, which might have led to lower effective exposure rates. It is possible that stronger frequency changes would have occurred if the experiment had been run for a longer period as additional individuals might have become infected. Other possible explanations include genetic variation in hosts or parasites (e.g., less susceptible

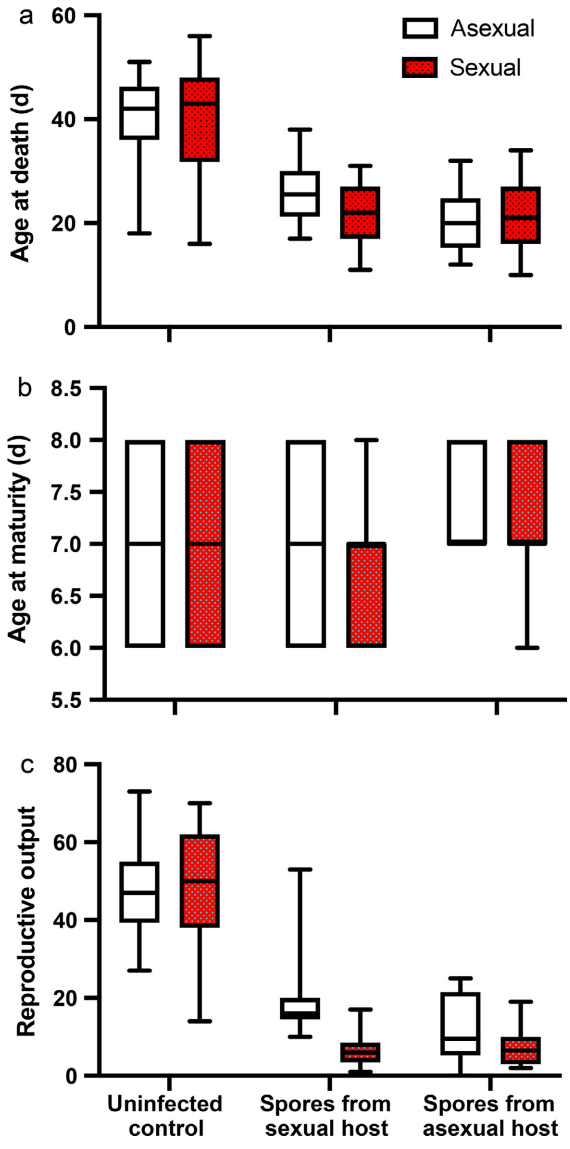

FIG. 4. Life history traits of asexual and sexual *Daphnia*, from either the control treatment, infection with spores of asexual origin treatment, or infection with spores of sexual origin treatment for (a) age at death, (b) age at first reproduction, and (c) reproductive output (number of offspring per mother). Box and whisker plots show the 25th to 75th percentiles with maximum and minimum values around the median.

sexuals or less infective parasites than in SK-39), and the fact that a part of the parasites were grown on asexuals for two years before the start of the experiment. These explanations seem, however, less likely, as the laboratory experiment also included hosts and parasites of diverse origin and nevertheless found strong effects.

Overall, our results show that parasites can lead to rapid changes in the ecological frequency dynamics of coexisting sexual and asexual taxa. Discussion on competition between sexuals and asexuals with regard to parasites is often framed within the Red Queen hypothesis, whereby asexuals are predicted to become overinfected with parasites (at least on average), as they cannot evolve as fast as their sexual competitors (Jaenike 1978, Lloyd 1980, Hamilton 1980, Hamilton et al. 1990). In contrast, here we find that asexuals replace sexuals (and not the other way around) as a consequence of a parasite-driven advantage. Yet, these results are still consistent with the Red Queen hypothesis. The hypothesis predicts that parasites should adapt to the most common genotypes, leading to a frequency-dependent advantage of rare genotypes (Jaenike 1978, Decaestecker et al. 2007, Salathé et al. 2008). Under this scenario, sexual reproduction is advantageous because it continually produces rare genotypes. However, when asexuals are rare as a whole group, parasites may adapt to sexuals, instead (Gibson et al. 2018). In Southern Finland, asexuals are relatively rare compared to sexuals (Lehto and Haag 2010), which may explain their advantage over sexuals in the presence of parasites. However, this advantage may only be transitory: once asexuals become abundant, parasites are predicted to adapt to them (Morran et al. 2011). Indeed, in our laboratory experiment, the spores obtained from asexual hosts were more virulent to asexuals than spores from sexual hosts. While this test was not replicated (we only tested one mixture of two parasite isolates from each of the two host types), this result suggests that some effects of parasite adaptation toward the new, asexual hosts may have started to become visible in our experiment. Finally, we cannot exclude that the higher asexual fitness is due to the adaptation of asexuals to the parasite. However, this seems less plausible given the lack of a shared long-term evolutionary history.

As outlined in the introduction, it is also possible that our results are indirectly linked to reproductive mode. Due to the invasive nature of the asexuals in this region, they do not share a long evolutionary history with the parasite, which is endemic to Europe (Green 1974, Friedrich et al. 1996) and may thus not have had time to adapt to the asexuals. Invasiveness may still be indirectly related to reproductive mode, as asexuals are overrepresented among invasive species (Ellstrand and Schierenbeck 2000, Sakai et al. 2001). Indeed, obligate asexual *Daphnia* have been shown to be effective invaders who have rapidly replaced resident populations of closely related sexuals in other parts of the world, not only in Finland (Mergeay et al. 2006, Fadda et al. 2011, Duggan et al. 2012, So et al. 2015). Even though reproductive assurance (Baker 1955) does not differ between sexual and asexual *Daphnia*, the success of obligate asexual *Daphnia* as invaders may still be linked to reproductive mode (though sexual *Daphnia* species can be invasive too; see Searle et al. 2016). First, obligate asexuality may allow a particularly successful invasive genotype to be "frozen" and thus shielded from segregation and mixing with other genotypes. In fact, what is essentially a single clone of *D. pulex* (or a group of closely related clones) is apparently responsible for the invasion of freshwater

habitats in Africa and Southern Europe (Mergeay et al. 2006, Fadda et al. 2011). Second, colonization by a single cyclical parthenogenetic individual leads to within-clone mating during the production of diapause stages, and within-clone mating is known to lead to strong inbreeding depression in this and other *Daphnia* species (Deng and Lynch 1997, Lohr and Haag 2015), also specifically with regard to parasites (Haag et al. 2003).

Our results suggest that parasites may have played an important role in the rapid establishment of these obligate asexual invaders, perhaps not only in Southern Finland but also elsewhere. In stark contrast, in the native range of asexual *D. pulex*, they are mostly outcompeted by sexuals if the two co-occur locally (Innes and Gin 2014). Though the possible involvement of parasites in the latter pattern is not known, this is potentially consistent with the "enemy release" advantage for invasive species, whereby invaders leave behind natural enemies from their native range and suffer less from newly encountered enemies in their introduced range (Keane and Crawley 2002, Torchin and Mitchell 2004, Blackburn and Ewen 2017).

Finally, it should not be forgotten that sexuals and asexuals often differ in many traits other than just the reproductive mode and that they often show ecological niche differentiation (e.g., Meirmans et al. 2012, Gilabert et al. 2014, Kotusz et al. 2014, Neiman et al 2018), including the sexuals and asexual studied here (Lehto and Haag 2010). In principle, this means that also factors independent of reproductive mode might have contributed to our results. Nonetheless, the rapid ecological replacement of sexuals by asexuals in the natural pond occurred in a manner consistent with a sometimes underappreciated aspect of the Red Queen, namely that asexuals can be at an advantage when they are rare and also with the invasion history of sexuals and asexuals studied here.

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