Supplementary Materials for

Genetic slippage after sex maintains diversity for parasite resistance in a natural host population

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**Results**

*Seasonal epidemics - Environment and ecology in the Aegelsee*

**Figure S1** Environmental conditions and sexual reproduction of *Daphnia magna* in the Aegelsee. **A**: Water temperature rise in the Aegelsee goes hand in hand with the appearance of the *Pasteuria ramosa* epidemics in the *D. magna* population. **Water temperature**: a temperature logger was installed in the pond from 2011 to 2018. No data is plotted in July 2013 because of vandalism of the data logger. The yearly temperature peaks in early October represent the release of warm ammoniacal condensation water in the pond. **Pasteuria prevalence**: red area plot represents *P. ramosa* prevalence in the *D. magna* population from 2011 to 2018. The grey horizontal line represents a water temperature threshold of 15 °C. When the water temperature rises above about 15 °C, the bacterial epidemics starts. **B**: Water level in the Aegelsee. Water level above sea level (a.s.l.) was read on a fixed floating device installed in the pond where the animals were sampled. We measured water level during the active season of the *D. magna*, from early April to early October. During this period, water level decreases progressively because of evaporation and agricultural and industrial use of the water. **C**: *Daphnia* species density in the Aegelsee, measured as the number of individuals per liter of water. We sampled the water column during the active season, from early April to early October. From 2011 to 2013, pond water was directly sampled in 1-L bottles. From 2014 on, a plankton net was used. These two protocols created a four-fold magnitude difference between values obtained in 2011-2013 and 2014-2018, which we represent on distinct y-axes. *Daphnia* species were subsequently determined in the laboratory. As *D. pulex* and *D. curvirostris* are difficult to tell apart, a subset of 100 individuals of these two species was used to infer their respective densities in 2014, 2016, 2017 and 2018. They are pooled in the other years. In early October, warm ammoniacal condensation water is released in the pond, killing all plankton. No *Daphnia* overwinter in this population, neither do resting stages hatch before early April. **D**: *D. magna* male production in the Aegelsee. We counted males in a subset of 100 *D. magna* at each collection point from 2016 to 2018. **E**: *D. magna* ephippia production in the Aegelsee. Five to nine sediment traps were installed on the pond floor and retrieved at each collection date in 2014, 2015, 2017 and 2018. The y-axis represents ephippia number relative to the total number of ephippia counted in the season.
Supplementary text S1 Environmental and ecological variables in the Aegelsee.

We observe cyclical changes in different environmental variables. Every year, the *Daphnia magna* population emerges when water temperature reaches about 12 °C (Fig. S1A). Temperature then increases to about 25 °C in summer, occasionally reaching peaks of 30 °C. In October, the warm ammonical condensation water is released in the pond, bringing temperature to 35−50 °C (Fig. S1A). The main increase in parasite prevalence occurs when water temperature rises above 15 °C (Fig. S1A). Water level in the Aegelsee is managed to make room for inflow of the condensation water in Fall. Therefore, every year the water level is lowered by about two meters over the course of the season. At its lowest level in late September, more than 80% of the pond sediments are exposed and the maximum water level is about one meter (Fig. S1B). *Daphnia* density shows irregular dynamics with a first peak typically in early summer, but further peaks may follow later. In most years, *D. magna* increases in relative frequency among all *Daphnia* species (Fig. S1C). We observe one or two peaks of *D. magna* male density during the season (Fig. S1D). We did not estimate the number of sexual females in the population, we instead collected resting stages in the sediment traps. Sexual egg counts cannot directly be compared with the frequencies of males, as they are time-shifted.

We observe a correlation between temperature cycles and *Pasteuria ramosa* epidemics in the *D. magna* Aegelsee population. Animals are observed to be infected by the bacteria as temperature rises above 15 °C every year in late April. We infer that epidemics are possibly influenced by water temperature, although the phenology of many other environmental factors may play a role. For example, longer day length, increased *Daphnia* density and lower water level (Fig. S1). It has been suggested that parasite-mediated selection in the *D. magna–P. ramosa* system is strongest at 20−25 °C (81, 82). Given climate change model predictions of pond warming and longer seasons with temperatures above 15 °C, selection for resistance can thus be expected to intensify in our study population. Warming could also affect the evolution of stress tolerance, as exposure to the pathogen disrupts the host’s ability to cope with thermal stress in this system (83). Environmental factors may also change the mode of selection: it has been shown that, under some temperature and food availability conditions, hosts in this system become more tolerant, thus potentially increasing parasite prevalence and slowing down coevolution (84). Parasite fitness may also be influenced by the interaction of genotype and environmental factors such as temperature and food availability (85), in a plant-parasite system: (86). Thus, while natural selection on resistance is precipitated on a high specificity of host-parasite interactions in the *D. magna–P. ramosa* system, it may also be linked to environmental conditions.

In the *D. magna–P. ramosa* system, host-parasite specificity is high, and spore attachment is not known to be influenced by environmental factors (51, 80). However, other host and parasite traits are influenced by the environment and there is intra-specific variability in how different genotypes respond to different environmental conditions (reviewed in (78)). Temperature was found to influence infectivity and spore production in the parasite in the present system (82, 85) and in the *D. dentifera–P. ramosa* system (87). In the host, temperature was found to influence virulence in the present system (81) and filtering rate and parasite prevalence in a *D. laevis–fungal* host-pathogen system (88–90). Temperature also increased epidemic size in two mesocosm experiments, in the present system (77) and in a *D. dentifera–fungal* parasite system (91). This was explained in the latter *Daphnia–fungus* system by an increase of the transmission rate, composed of infectivity and foraging rate (92). Nutrient availability increased tolerance of *D. magna* to *P. ramosa*, and increased spore production in the parasite, irrespective of temperature variations (84). Nutrient availability has also been shown to have a differential impact on fecundity and survival in distinct *D. magna* genotypes, leading to differential consequences of infection by a viral parasite (93). Epidemiological variables such as prevalence, virulence, transmission rate and infection rate are thereby shaped by environmental variables (94).
Selection and sexual reproduction

Figures S2 to S4: The *Daphnia magna* overwintering resting stages in the Aegelsee

The *Daphnia magna* population in the Aegelsee goes through a cyclical pattern of resistotype (resistance phenotype) frequency. Resistant phenotypes increase in frequency over the course of the epidemics but resistotype diversity is created anew each spring via the hatching of the resting stages overwintering population. We collected and hatched ephippia laid in the water column by the planktonic population of *D. magna* throughout the active season in 2014, 2015, 2017 and 2018 using sediment traps (Figs. S2). We subsequently collected and hatched ephippia from surface sediment in winter 2014 as a representative sample of the spring *D. magna* cohort (Fig. S3). Figure S4 represents planktonic, ephippia and hatchling data together as a timeseries.
Figure S2 Hatching of the *Daphnia magna* overwintering resting stages in the Aegelsee. Ephippia were collected in 2014, 2015, 2017 and 2018 during the active season. We used five to nine sediment traps at each collection date. Ephippia were subsequently stored at 4 °C to mimic the resting period and hatching was induced the following spring by placing the ephippia in outside containers. In 2014, the first ephippia sample was lost. In 2015, no ephippia hatched from the last sample because of exposure to the warm ammoniacal condensation water released into the pond at the end of the season.

**A**: Relative proportion of ephippia laid in the water column during the active season of *D. magna*. **B** and **C**: Number of resting stages per ephippium. On a subset of ephippia not used in the hatching experiment, we counted the number of resting stages present in the ephippial case. In ephippia collected in 2014, egg number was counted after the hatching experiment. This is why we do not plot it here, but we did use the values to infer hatching success (see E below). We checked ten to 20 ephippia for a minimum of two repeats at each collection date. **D**: Number of resting stages per ephippium, as the proportion of ephippia containing zero, one or two resting stages. **E**: Number of resting stages per ephippium, as the mean number of resting stages in the ephippial case. **D**: Hatching rate and pattern of collected ephippia. We induced hatching of the collected resting stages by putting 20 to 100 ephippia; depending on how many were collected; of each repeat of each collection date in outside containers. Resting stages collected in 2014, 2015 and 2017 were induced on 18 March 2015, 11 April 2016 and 29 March 2018, respectively. Hatching was monitored every second day. Vertical graphs represent the relative proportion of hatchlings over time after hatching induction. Each vertical graph represents ephippia from each collection date during the season. The different repeats for each collection date are pooled in one vertical graph. The y-axis (x-axis of the vertical graphs) is the number of days since hatching induction. In 2017, the total proportion of hatchlings across repeats and collection dates is presented. Resting stages collected on the last collection date of 2015 did not hatch because the warm ammoniacal condensation water had been released before our sampling. Variation across years might be due to the different hatching induction dates in the different years. **E**: Hatching success. We calculated hatching rate for each repeat for each collection date. We used the total number of hatchlings and the total number of resting stages induced, inferred from the subset of ephippia opened (B and C). For resting stages collected in 2014, only the number of remaining resting stages after hatching induction was counted. We inferred the total number of induced resting stages by adding the number of remaining resting stages to the total number of hatchlings. **F**: Resistotype (resistance phenotype) of hatched animals. Hatchlings were cloned in the laboratory to assess their resistotype. We tested 20 *D. magna* clonal lines (clones) for each repeat for each collection date, resulting in about 100 clones for each collection date.
Daphnia magna overwintering resting stages in the Aegelsee, collected in the sediment in winter 2014. The overwintering resting stages in winter 2014 were laid in the active season in 2013 and reflects the spring 2014 D. magna cohort. We collected five replicates of surface sediment in the pond in February 2014, before onset of the natural hatching season. A hundred ephippia from each replicate were placed in outdoor containers in late February 2014 and hatching was monitored every second day.

A: Number of hatchlings over time after hatching induction. The five replicates are represented in different shades of grey. A total of 608 hatchlings were recorded.

B: Resistotype (resistance phenotype) distribution of hatchlings over time after hatching induction. Hatchlings were put separately in jars to produce clonal lines. We measured resistotype on a subset of 381 randomly chosen clones. We represent resistotype frequency of hatched animals in three date intervals because of low sample sizes at some dates. The x-axis in A and B spans from day 0, the 20 February 2014 to day 103, the 3 June 2014.

C: total resistotype proportions resulting from all 381 hatchlings.

D: resistotype frequency of sampled D. magna in 2014.
Figure S4  *Daphnia magna* overwintering resting stages in the Aegelsee, collected in the water column throughout the active season. The spring *D. magna* cohort hatches from the resting stages present in the pond sediment. Throughout the active season (from early April to early October), *D. magna* reproduce asexually (clonal eggs) and sexually (fertilized resting stages). The resting stages create the overwintering population. In early October, warm condensation ammoniacal water is released in the pond, killing all plankton but not the resting stages. In winter, no ephippia hatch. **Planktonic:** Resistotype frequency in the *D. magna* population from 2014 to 2018. A large batch of animals was collected from early April to early October every 2−4 weeks to clone about 60 to 100 females. The resistotype is the full resistance phenotype to five *Pasteuria ramosa* isolates: C1, C19, P15, P20 and P21. Resistance and susceptibility are denoted as R and S, respectively. Note that in different years, different numbers of *P. ramosa* isolates were tested. We use the placeholder "⎵" in the resistotype when a bacterial isolate was not tested. Resistance to P20 is highlighted because of its importance in the evolution of the host population (50). **Ephippia:** Relative number of *D. magna* ephippia laid in the pond. Five to nine ephippia traps were set up in 2014, 2015, 2017 and 2018 and collected every 2−4 weeks from early April to early October. We plot the mean number of ephippia per trap at each timepoint divided by the total mean number of ephippia laid during the whole year. Time on the x-axis represents the middle point between setup and collection of the traps. **Hatching:** *D. magna* resting stages collected in 2014, 2015 and 2017 were hatched in outside containers the following spring, after a resting period at 4 °C. In each container, 20 to 100 ephippia per trap per timepoint were placed, depending on how many were collected. Hatched animals were cloned in the laboratory. In *Daphnia*, hatchlings from resting stages are female, which allows to clone them. We measured the resistance phenotype (resistotype) of 20 clones per trap per timepoint, resulting in 100 clones per timepoint. **Weighted sum:** Weighted sum of *D. magna* resistotype frequency from hatched ephippia. Total *D. magna* resistotype frequency from hatched ephippia weighted by the relative number of ephippia laid at each timepoint. The weighted sum of resistotype frequency represents the overwintering resting stages. In 2014, the first ephippia sample was lost. In 2015, no ephippia hatched from the last sample because of exposure to the warm ammoniacal condensation water released into the pond at the end of the season.
Calculation of expected resistotype frequencies in resting stages

Figure S5 Genetic model of resistance in the Aegelsee. The model includes resistotypes to C1, C19, P15 and P20 Pasteuria ramosa isolates. Resistance to C1 and C19 determined by the ABC cluster was described in (37). The dominant allele at the B locus induces resistance (R) to C19 and susceptibility (S) to C1. The dominant allele at the C locus confers resistance to both C1 and C19 P. ramosa isolates, regardless of the genotype at the B locus. Variation at the A locus is not considered here as the recessive allele at this locus is believed to be fixed in the population (50). The D locus determines resistance to P15 (55). The E locus determines resistance to P20 (50). Resistance is dominant at the B and C loci (resistance to C1 and C19) whereas resistance is recessive at the D and E loci (resistance to P15 and P20, respectively). Recessive homozygosity at the B and C locus induces susceptibility to P20, regardless of the genotype at the E locus (50). Hence the epistasis can only be observed phenotypically in "bbccce" (SS_S) offspring. If the epistatic relationship is not present in this case, the observed phenotype would be SS_R. Such SS_R individuals were never observed in the population. Resistotypes determined by the "B-" and the "dd" genotype, regardless of the genotype at other loci, are very rare or do not occur in the D. magna population. We assume the B and the d alleles are rare in the population and might induce poor fitness. We implement this genetic model of resistance in the D. magna–P. ramosa system in the "peas" R package (Doc. S1). We subsequently test the model using resting stages hatching data (Figs. S6 and S7, Tables S1 and S2).
Using the genetic model of resistance inheritance in the *Daphnia magna*–*Pasteuria ramosa* system, we calculate predicted resistotype frequency resulting from hatching of *D. magna* resting stages, or ephippia, produced throughout the active season. We compare this expected resistotype frequency to the observed resistotype frequency obtained from hatching of field-collected resting stages. The genetic model and calculations are described in Fig. S5, Doc. S1, Doc. S2 and Fig. S8. In short, we use three input datasets: (i) the longitudinally observed resistotype frequency, from the F0 generation performing sexual reproduction during the active season, (ii) the predicted F1 resistotype segregation given by the genetic model and (iii) the genotype distribution within resistotypes in the *D. magna* population, the F0 generation, described here. Because we use phenotype distribution data, we input genotype distribution within each phenotype. Figs. S6 and S7: observed vs. expected resistotype frequency resulting from resting stages hatching. We calculate expected resistotype frequency according to different allele frequency scenarios in the *D. magna* population. Tables S1 and S2: Allele frequency scenarios in the *D. magna* population. We use the list of possible genotypes and their corresponding resistotypes given by the genetic model of resistance in the system. In each scenario, we fix an allele at one or several loci and we equally distribute genotype proportions among the other loci. In resistotypes where it is not possible to fix the allele, we equally distribute genotype proportions among heterozygous genotypes or among homozygous genotypes for the alternative allele when this is the only possible genotype determining the resistotype. Table S2 presents the “bbDD” scenario, additionally implemented with observed allele frequency at the C and E loci. Observed C and E loci allele frequency were measured in spring sample (50).

- **scenario “bbDD”:** the “bb” and “DD” genotypes are fixed in all possible resistotypes.
- **scenario “bb”:** the “bb” genotype is fixed in all possible resistotypes.
- **scenario “BB”:** the “BB” genotype is fixed in all possible resistotypes.
- **scenario “CC”:** the “CC” genotype is fixed in all possible resistotypes.
- **scenario “DD”:** the “DD” genotype is fixed in all possible resistotypes.
- **scenario “ee”:** the “ee” genotype is fixed in all possible resistotypes.
- **scenario “EE”:** the “EE” genotype is fixed in all possible resistotypes.
- **scenario “hetero”:** all loci show heterozygous genotype in all possible resistotypes.

*Note:* we use four-letter resistotype because the genetic model of resistance includes resistance to the four *P. ramosa* isolates C1, C19, P15 and P20.
Figure S6. Expected resistotype frequency resulting from resting stages, or ephippia, laid throughout the active season of *Daphnia magna*. We use the genetic model of resistance inheritance in the *Daphnia magna*–*Pasteuria ramosa* system, presented in Fig. S5. Allele frequency scenarios are detailed in Table S1.
Figure S7  Expected resistotype frequency resulting from resting stages produced throughout the active season of *Daphnia magna*. We use the genetic model of resistance inheritance in the *Daphnia magna*–*Pasteuria ramosa* system. This genetic model, presented in Fig. S5, includes four loci with dominance and epistasis controlling resistance to the four *P. ramosa* isolates C1, C19, P15 and P20. The last panel presents the expected resistotype distribution fitting best the observed one. We calculated it under the “bbDD” scenario, where the b and D alleles are fixed in all possible phenotypes, and where allele frequencies at the C and E loci are inferred from measured frequencies in spring 2015. This scenario is detailed in Table S2.
Table S1: Genotype distribution scenarios in the Aeglessee Daphnia magna population. In each scenario we fix an allele at one or two loci and we equally distribute proportions in the remaining possible genotypes, within each resistotype. In resistotypes where it is not possible to fix the allele, we equally distribute genotype proportions among heterozygous genotypes or among homozygous genotypes for the alternative allele when this is the only possible genotype determining the resistotype. The genetic model described in Fig. S5 provided the list of possible genotypes and their corresponding resistotypes. The different scenarios are described above. This table corresponds to the “freq” table described in Supplementary Doc. S2 and Fig. S8.

| pheno | geno | Scenarios of genotype distribution within resistotypes |
|-------|------|-----------------------------------------------------|
|       |      | bbDD | bb | BB | CC | CC | dd | DD | ee | EE | hetero |
| RRRR  | RRRR | 1/2  | 1/2 | 1/3 | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 1 |
| RRRR  | RRRR | 0   | 0   | 0   | 1/3 | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 1 |
| RRRR  | RRRR | 1/2  | 1/2 | 1/3 | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 0 |
| RRRR  | RRRR | 0   | 0   | 0   | 1/3 | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 0 |
| RRRR  | RRRR | 1/2  | 1/2 | 0   | 1/3 | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 0 |
| RRRR  | RRRR | 0   | 0   | 0   | 0   | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 0 |
| RRRR  | RRRR | 1/2  | 1/2 | 1/3 | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 0 |
| RRRR  | RRRR | 0   | 0   | 0   | 0   | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 0 |
| RRRR  | RRRR | 1/2  | 1/2 | 0   | 0   | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 0 |
| RRRR  | RRRR | 0   | 0   | 0   | 0   | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 0 |
| RRRR  | RRRR | 1/2  | 1/2 | 0   | 0   | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 0 |
| RRRR  | RRRR | 0   | 0   | 0   | 0   | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 0 |
| RRRR  | RRRR | 1/2  | 1/2 | 1/3 | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 0 |
| RRRR  | RRRR | 0   | 0   | 0   | 0   | 1/6 | 1/6 | 1/6 | 1/6 | 1/6 | 0 |

Note: The table continues with similar scenarios for different combinations of alleles and resistotypes.
Table S2: Resistance genotype distribution scenario in the Aegelsee Daphnia magna population, producing an expected resistotype distribution that fits best the observed one. We fix the “b” and the “D” alleles, and we distribute proportions in the remaining possible genotypes, within each resistotype, using C and E loci allele frequency observed in spring 2015. In resistotypes where it is not possible to fix the allele, we equally distribute genotype proportions among heterozygous genotypes or among homozygous genotypes for the alternative allele when this is the only possible genotype determining the resistotype. The genetic model described in Fig. S5 provided the list of possible genotypes and their corresponding resistotypes. This table corresponds to the "freq" table described in Supplementary Doc. S2 and Fig. S8.

| pheno geno | Genotype distribution within resistotypes | bbDD scenario and C- and E-loci allele frequency inferred |
|------------|------------------------------------------|-------------------------------------------------------|
| BBCC      | X                                         | f_{bbCC}(CC)=f(CC)/((f(CC)+f(Cc)*(f(Ee)+f(EE)))=0.052 |
| BBCC      | X                                         | f_{bbCC}(CC)=f(CC)/(f(CC)+f(EE))=0.557                |
| BBcc      | X                                         | f_{bbcc}(CC)=f(CC)=0.35                              |
| BBcc      | X                                         | f_{bbcc}(CC)=f(CC)/(f(CC)+f(Ee))=0.052                |
| BBCC      | X                                         | f_{bbCC}(CC)=f(CC)=0.35                              |
| BBCC      | X                                         | f_{bbCC}(CC)=f(CC)/(f(CC)+f(Ee))=0.052                |
| BBcc      | X                                         | f_{bbcc}(CC)=f(CC)=0.35                              |
| BBcc      | X                                         | f_{bbcc}(CC)=f(CC)/(f(CC)+f(Ee))=0.052                |
| BBCC      | X                                         | f_{bbCC}(CC)=f(CC)=0.35                              |
| BBCC      | X                                         | f_{bbCC}(CC)=f(CC)/(f(CC)+f(Ee))=0.052                |
| BBcc      | X                                         | f_{bbcc}(CC)=f(CC)=0.35                              |
| BBcc      | X                                         | f_{bbcc}(CC)=f(CC)/(f(CC)+f(Ee))=0.052                |
| BBCC      | X                                         | f_{bbCC}(CC)=f(CC)=0.35                              |
| BBCC      | X                                         | f_{bbCC}(CC)=f(CC)/(f(CC)+f(Ee))=0.052                |
| BBcc      | X                                         | f_{bbcc}(CC)=f(CC)=0.35                              |
| BBcc      | X                                         | f_{bbcc}(CC)=f(CC)/(f(CC)+f(Ee))=0.052                |

Doc. S2 and Fig. S5.
Figure S8 Participation of the different resistotypes to sexual reproduction in the host population. To assess whether sexual reproduction was biased towards some resistotypes, we collected in August 2020 *Daphnia magna* samples and quantified the resistotype distribution of females carrying resting stages, of males and of a random sample of females. We then compare this to a random sample of females in the spring 2021, which represents the result of sexual recombination from sexual eggs produced in the previous year. Because males cannot be cloned, we scored their resistotype by assessing attachment to all parasite isolates successively on the same individual. After scoring the first isolate, we transferred the animal in clean water to allow for molting which removes the parasite spores. We hence cannot produce replicates, resulting in higher uncertainty of their resistotypes, in particular for the less easily scored P15 and P21 *Pasteuria ramosa* isolates, which attach to a different part of the gut than the other isolates.
Discussion

**Supplementary text S2 Are our results case-specific?**

Genetic slippage has rarely been described and never in a natural population. The here presented results may therefore seem to be a special case. We believe that this is not the case, but that the biology of the system in combination with the unique setting of our study population allowed us to clearly observe the effect of selection and slippage particularly. Several features of the system made this possible: (i) we study an ecologically relevant trait that is easy to score in many host individuals using the attachment test, (ii) expression of this trait is, to the best of our knowledge, independent of the environment (51, 95). Any other mendelian trait under selection would work equally well, while quantitative traits might be harder to study in this respect, as they are often sensitive to the environment (47). In our system, the phenotypic changes were thus tractable with high accuracy and high throughput. The field site is unique in that the pond is used in winter as a sewage pond, killing all planktonic animals. Each spring, only hatchlings from sexual resting eggs form the new population. The population undergoes yearly epidemics of the bacterial parasite Pasteuria ramosa, which allowed us to monitor cyclical selection by a parasite in the host population. The absence of predatory fish and near absence of invertebrate predators in this population (50) may promote these P. ramosa epidemics, as predation is known to reduce rates of parasitism in Daphnia populations (96). The asexual reproduction of the host during the planktonic season makes the effect of selection standing out very strongly. Genetic slippage can also be observed in normal sexual species, however less strongly. Many other cyclic parthenogenetic species exist in habitats with similar properties, inviting for studies with a similar design as ours to explore the generality of genetic slippage.
Methods

Hatching modelling

Document S1 peas implementation of the genetic model of resistance in the Daphnia magna–Pasteuria ramosa system.

**Genetic model for resistance in the Aegelsee**
implemented in peas R package

**BCDE genetic model**

1. **Install the package**
   #install.packages("devtools")
   #devtools::install_github("JanEngelstaedter/peas", build_vignettes = TRUE)

   library(peas)

2. **Set up the genetic model**

2.1 **Defining the genetic system**
   \[
   r^2 = \frac{1-\exp(-2 \times 23.1/100)}{2} \quad \# \text{recombination rate between the B and C loci (Metzger et al. 2016)}
   \]

   \# 4 loci with 2 alleles each, BC clustered together (Metzger et al. 2016).
   BCDE <- newGenopheno(nloci = 2,
                       alleleNames = list(c("b", "B"). c("c", "C").
                       rec = r2)
   BCDE <- addLinkageGroup(BCDE, alleleNames = list(c("d", "D").)
   BCDE <- addLinkageGroup(BCDE, alleleNames = list(c("e", "E").)

2.2 **Set genotypes and their corresponding phenotypes : THE GENETIC MODEL**

   BCDE <- setPhenotypes(BCDE, "S/R", "__~__ | __ | __","SSRR") \# default all receive --> SSRR
   \# (we don’t take the epistasis relation into account, this will be the last line of the model)

   BCDE <- setPhenotypes(BCDE, "S/R", "B_~__ | __ | __","SRRR") \# B --> R to C19

   BCDE <- setPhenotypes(BCDE, "S/R", "B_~__ | D_ | __","SRSR") \# D --> S to P15
   \# (Bento et al. 2020)
   BCDE <- setPhenotypes(BCDE, "S/R", "B_~__ | E_","SRRS") \# E --> S to P20
   BCDE <- setPhenotypes(BCDE, "S/R", "B_~__ | D_ | E_","SRSS") \#

   BCDE <- setPhenotypes(BCDE, "S/R", "__C_ | __ | __","RRRR") \# C hides B and --> R to C1 and C1
BCDE <- `setPhenotypes`(BCDE, "S/R", "__~C_|D_|__", "RRSR") #
BCDE <- `setPhenotypes`(BCDE, "S/R", "__~C_|E_", "RRRS") #
BCDE <- `setPhenotypes`(BCDE, "S/R", "__~C_|D_|E_", "RRSS") #

# b/c-E epistasis #
# "bbcc" genotype induces S to P20, regardless of genotype at E-locus
BCDE <- `setPhenotypes`(BCDE, "S/R", "bb~cc|D_|__", "SSSS") #
BCDE <- `setPhenotypes`(BCDE, "S/R", "bb~cc|dd|__", "SSRS") #

Summary of model
BCDE
## Genetic system comprising 3 linkage groups:
## Linkage group 1: autosomal, 2 loci with recombination rate 0.1849888
## Alleles at locus 1: b, B
## Alleles at locus 2: c, C
## Linkage group 2: autosomal, 1 locus
## Alleles at locus 1: d, D
## Linkage group 3: autosomal, 1 locus
## Alleles at locus 1: e, E
## Phenotypes defined for the following traits:
## S/R (trait values: SSRS, SRRR, RRRR, SSSS, SRSR, RRSR, SRSS, RRSS, SRSS, RRSS)

List of all possible genotype combinations and corresponding phenotypes
BCDEallgeno<-`getPhenotypes`(BCDE)
nrow(BCDEallgeno) # 81 possible genotypes

## [1] 81
nrow(unique(BCDEallgeno)) # 10 possible resistotypes

## [1] 10

BCDEallgeno$geno<-`row.names`(BCDEallgeno) # add "geno" column
BCDEallgeno<-BCDEallgeno[order(BCDEallgeno$S/R),] # sort resistotypes
# rename "S/R" column as "pheno"
library(tidyverse)
BCDEallgeno<-`rename`(BCDEallgeno, pheno=`S/R`)
# export in xl file
library(xlsx)
write.xlsx(BCDEallgeno,"BCDEallgeno.xlsx")

3. Predict crosses
# all possible genotype crossings and their
# expected F1 genotype and phenotype segregation

cross<-`matrix`(list(), nrow=nrow(BCDEallgeno), ncol=nrow(BCDEallgeno), byrow=T)

for (i in 1:nrow(BCDEallgeno)) {
for (j in 1:nrow(BCDEallgeno)) {
    cross[[i,j]]< predictCross(BCDE, BCDEallgeno$geno[i], BCDEallgeno$geno[j])
    # we add the corresponding resistotypes to the genotype output
    cross[[i,j]]$genotypes$SRtrait <- getPhenotypes(BCDE, equivalent = "none")[row.names(cross[[i,j]]$genotypes),]
}
}

# large matrix of 81*81=6561 elements
# example: cross between genotype#1 and genotype#2
BCDEallgeno[1,]

## pheno geno
## bb~Cc | dd | ee  RRRR bb~Cc | dd | ee
BCDEallgeno[2,]

## pheno geno
## Bb~Cc | dd | ee  RRRR Bb~Cc | dd | ee

cross[[1,2]]

## $genotypes
## fraction SRtrait
## bb~CC | dd | ee 0.20375279 RRRR
## bb~CC | dd | ee 0.04624721 RRRR
## bB~Cc | dd | ee 0.25000000 RRRR
## bb~Cc | dd | ee 0.25000000 RRRR
## bB~cc | dd | ee 0.04624721 SRRR
## bb~cc | dd | ee 0.20375279 SSRS
#
## $phenotypes
## S/R fraction
## 1 RRRR 0.75000000
## 2 SRRR 0.04624721
## 3 SSRS 0.20375279

# find a genotype
match("Bb~cc | dd | EE", BCDEallgeno$geno) # position 59

## [1] 59
**Doc. S2 and Fig. S9:** Theoretical resistotype frequency resulting from ephippia hatching in the Aegelsee.

Using the genetic model of resistance in the *Daphnia magna*–*Pasteuria ramosa* system, we calculate theoretical resistotype (resistance phenotype) frequency resulting from hatching of *D. magna* resting stages laid throughout the active season.

*Note:* we use four-letter resistotype because the genetic model of resistance includes resistance to the four *P. ramosa* isolates C1, C19, P15 and P20.

**Document S2** calculations of the theoretical resistance phenotype frequency resulting from ephippia hatching in the Aegelsee.

**prop data frame:** observed resistotype proportion in the study population at each sampling date.

| prop | date-1 | date-2 | date-3 | ... | date-i | date-d |
|------|--------|--------|--------|-----|--------|--------|
| pheno-1 (RRRR) | p1,1 | p1,1 | p2,1 | ... | p_i,1 | p_d,1 |
| pheno-k | ... | p1,k | ... | ... | ... | ... |
| pheno-n (SSSS) | ... | ... | ... | ... | p_i,n | ... |

**freq data frame:** fraction of each possible genotype within each phenotype. We use the list of possible genotypes and their corresponding phenotypes given by the genetic model of resistance implemented in the "peas" R-package. This list is given by the "getPhenotypes" function in the "peas" R-package (Doc. S1). The fraction of each possible genotype within each phenotype (fq) is implemented by the user. In Figs. S6 and S7, Tables S1 and S2 we test different scenarios of genotype distribution within the resistotypes.

| freq | pheno | geno | fq |
|------|-------|------|----|
| pheno-1 (RRRR) | gene-1 | f1,1 |
| pheno-1 (RRRR) | gene-2 | f2,1 |
| pheno-1 (RRRR) | gene-3 | f3,1 |
| pheno-2 | gene-4 | f4,2 |
| pheno-2 | gene-5 | f5,2 |
| pheno-2 | gene-6 | f6,2 |
| ... | ... | ... |
| pheno-k | ... | ... |
| pheno-k | ... | ... |
| pheno-k | gene-j | f_j,2 |
| ... | ... | ... |
| pheno-n (SSSS) | gene-g | f_g,n |

We then calculate the proportion of each genotype at each sampling date:

\[ d_{j,i} = f_{j,k} \times p_{i,k} \]

with
\(d_{ij}\): proportion of the \(j\)-genotype at the \(i\)-date.

\(f_{jk}\) from the freq data frame: proportion of the \(j\)-genotype within the \(k\)-phenotype. This is implemented by the user.

\(p_{ik}\) from the prop data frame: proportion of the \(k\)-phenotype at the \(i\)-date. Input from sampled data in the study population.

freq

| pheno | geno | f1 | date-1 | date-2 | ... | date-4 | ... | date-d |
|-------|------|----|--------|--------|-----|--------|-----|--------|
| pheno-1 | geno-1 | f_{11} | d_{11} | d_{12} | ... | d_{14} | ... | d_{1d} |
| pheno-1 | geno-2 | f_{11} | d_{21} | d_{22} | ... | d_{24} | ... | d_{2d} |
| pheno-1 | geno-3 | f_{11} | d_{31} | d_{32} | ... | d_{34} | ... | d_{3d} |
| ... | ... | ... | ... | ... | ... | ... | ... | ... |
| pheno-k | ... | ... | ... | ... | ... | ... | ... | ... |
| pheno-k | ... | ... | ... | ... | ... | ... | ... | ... |
| pheno-k | ... | ... | ... | ... | ... | ... | ... | ... |
| pheno-n | ... | ... | ... | ... | ... | ... | ... | ... |

\[ \begin{pmatrix} d_{1,i} \\ d_{2,i} \\ \vdots \\ d_{j,i} \\ \vdots \\ d_{g,i} \end{pmatrix} \cdot t \begin{pmatrix} d_{1,i} \\ d_{2,i} \\ \vdots \\ d_{j,i} \\ \vdots \\ d_{g,i} \end{pmatrix} = \begin{pmatrix} d_{1,1} & d_{1,2} & \cdots & d_{1,m} & \cdots & d_{1,g} \\ d_{2,1} & d_{2,2} & \cdots & d_{2,m} & \cdots & d_{2,g} \\ \vdots & \vdots & \ddots & \vdots & \ddots & \vdots \\ d_{j,1} & d_{j,2} & \cdots & d_{j,m} & \cdots & d_{j,g} \\ \vdots & \vdots & \ddots & \vdots & \ddots & \vdots \\ d_{g,1} & d_{g,2} & \cdots & d_{g,m} & \cdots & d_{g,g} \end{pmatrix} \]

with

\(d_{ijm}\): proportion of mating events between the \(l\)-genotype and \(m\)-genotype at the \(i\)-date.

The sum of the elements of the di matrix is equal to 1.

cross matrix

\[ \begin{pmatrix} c_{1,1} & c_{1,2} & \cdots & c_{1,m} & \cdots & c_{1,g} \\ c_{2,1} & c_{2,2} & \cdots & c_{2,m} & \cdots & c_{2,g} \\ \vdots & \vdots & \ddots & \vdots & \ddots & \vdots \\ c_{l,1} & c_{l,2} & \cdots & c_{l,m} & \cdots & c_{l,g} \\ \vdots & \vdots & \ddots & \vdots & \ddots & \vdots \\ c_{g,1} & c_{g,2} & \cdots & c_{g,m} & \cdots & c_{g,g} \end{pmatrix} \]

\(c_{ilm}\): data frame containing predicted genotypic and phenotypic crossing results between the \(l\)- and the \(m\)-genotype. This was calculated using the "predictCross" function in the "peas" R package. See implementation of the genetic model of resistance in Doc. S1.

Example:

\(c_{1,2}\): crossing result between genotype \#1 and genotype \#2

BCDEallgeno[1.]
### pheno geno
### bb~Cc | dd | ee  RRRR bb~Cc | dd | ee

BCDEallgeno[2,]

### pheno geno
### Bb~Cc | dd | ee  RRRR Bb~Cc | dd | ee
cross[[1,2]]

### $genotypes
### fraction SRtrait
### bB~CC | dd | ee 0.20375279
### bb~CC | dd | ee 0.04624721
### bB~Cc | dd | ee 0.25000000
### bB~cc | dd | ee 0.04624721
### bb~cc | dd | ee 0.20375279

### $phenotypes
### S/R fraction
### 1 RRRR 0.75000000
### 2 SRRR 0.04624721
### 3 SSRS 0.20375279

With y ≤ x as there can be several genotypes underlying one phenotype (see example above)

### c\text{lm}$genotypes

| geno-α | fraction | SRtrait |
|--------|----------|---------|
| geno-a | α_a      | pheno-a |
| geno-b | α_b      | pheno-b |
| geno-c | α_c      | pheno-y |
| ...    | ...      | ...     |

### prophatch data frame: final data frame with expected resistotype proportions at each sampling date.

### prophatch

| date-1 | pheno-1 (RRRR) | ... | pheno-k | ... | pheno-n (SSSS) |
|--------|----------------|-----|---------|-----|----------------|
| date-2 | h_{1,1}        | ... | h_{1,k} | ... | h_{1,n}        |
| date-3 | h_{2,1}        | ... | h_{2,k} | ... | h_{2,n}        |
| ...    | ...            | ... | ...     | ... | ...            |
| date-i | h_{i,1}        | ... | h_{i,k} | ... | h_{i,n}        |
| ...    | ...            | ... | ...     | ... | ...            |
| date-d | h_{d,1}        | ... | h_{d,k} | ... | h_{d,n}        |

h_{i,k}: expected frequency of the k-resistotype at the i-date.

\[ h_{i,k} = \sum_{m=0}^{j} \sum_{l=0}^{j} c_{l,m}$phenotypes$\beta_{k} \ast d_{l,m} \]

The same calculation can be done with the genotypes fractions (α) instead of the phenotypes fractions (β).
Figure S9 Summary illustration of Doc. S2: calculation of theoretical resistotype (resistance phenotype) frequency resulting from resting stages hatching in the Aegelsee *Daphnia magna* population. The theoretical resistotype frequency over time is calculated using the genetic model of resistance in the *D. magna−Pasteuria ramosa* system and the observed resistotype frequency over time in the *D. magna* population. Input elements are written in regular font style, output elements are written in bold. **Prop** data frame: observed resistotype proportion in the study population at each sampling date. **Freq** data frame: fraction of each possible genotype within each phenotype. We use the list of possible genotypes and their corresponding phenotypes given by the genetic model of resistance implemented in the "peas" R-package. **Di** matrix: calculated from "prop" and "freq": fraction of all possible genotype crossings at the i-date considering random mating in the *D. magna* population. **Cross** matrix: predicted genotypic and phenotypic crossing results from all possible genotype crossings. Each element of the matrix is a data frame containing predicted genotypic and phenotypic crossing results of one genotype crossing. **Prophatch** data frame: calculated from "di" and "cross": final data frame with expected resistotype proportions resulting from sexual reproduction at each sampling date.

Document S3 Statistical software.

Unless otherwise stated, all statistical analyses and graphics were performed in the R software version 3.6.1 (http://www.R-project.org). Graphics were edited in Inkscape v. 1.0.1 (https://inkscape.org/). Mean values are presented with standard error: mean ± se (Package RVAideMemoire v. 0.9-45-2 (http://CRAN.R-project.org/package=RVAideMemoire)). Packages used in R for package installation, data manipulation and graphics are the following: package development, documentation and installation: devtools v. 2.2.1 (https://CRAN.R-project.org/package=devtools) and roxygen2 v. 6.1.1 (https://CRAN.R-project.org/package=roxygen2), data manipulation: dplyr v. 0.8.3 (https://CRAN.R-project.org/package=dplyr), tidyverse v. 1.0.0 (https://CRAN.R-project.org/package=tidyr), tidyquant v. 0.5.8 (https://CRAN.R-project.org/package=tidyquant), tidyverse v. 1.2.1 (https://CRAN.R-project.org/package=tidyverse), xlsx v. 0.6.1 (https://CRAN.R-project.org/package=xlsx), ggplot2 v. 3.3.0 (https://CRAN.R-project.org/package=ggplot2), extrafont v. 0.17 (https://CRAN.R-project.org/package=extrafont), scales v. 1.0.0 (https://CRAN.R-project.org/package=scales), cowplot v. 1.0.0 (https://CRAN.R-project.org/package=cowplot), gridExtra v. 2.3 (https://CRAN.R-project.org/package=gridExtra), ggpubr v. 0.2.3 (https://CRAN.R-project.org/package=ggpubr), ggplotify v. 0.0.4 (https://CRAN.R-project.org/package=ggplotify), magick v. 2.2 (https://CRAN.R-project.org/package=magick), ggsci v. 2.9 (https://CRAN.R-project.org/package=ggsci) and png v. 0.1.7 (https://CRAN.R-project.org/package=png).
REFERENCES AND NOTES

1. R. A. Fisher, *The Genetical Theory of Natural Selection* (Oxford Univ. Press, 1930).

2. R. A. Fisher, Polymorphism and natural selection. *J. Ecol.* **46**, 289–293 (1958).

3. S. A. Frank, Wright’s adaptive landscape versus Fisher’s fundamental theorem, in *The Adaptive Landscape in Evolutionary Biology*, E. Svensson, R. Calsbeek, Eds. (Oxford Univ. Press, 2013), pp. 41–57.

4. A. D. Kern, M. W. Hahn, The neutral theory in light of natural selection. *Mol. Biol. Evol.* **35**, 1366–1371 (2018).

5. K. J. M. Jeffery, C. R. M. Bangham, Do infectious diseases drive MHC diversity? *Microbes Infect.* **2**, 1335–1341 (2000).

6. A. L. Hughes, Natural selection and the diversification of vertebrate immune effectors. *Immunol. Rev.* **190**, 161–168 (2002).

7. J. Radwan, W. Babik, J. Kaufman, T. L. Lenz, J. Winternitz, Advances in the evolutionary understanding of MHC polymorphism. *Trends Genet.* **36**, 298–311 (2020).

8. S. Sommer, The importance of immune gene variability (MHC) in evolutionary ecology and conservation. *Front. Zool.* **2**, 16 (2005).

9. E. Baggs, G. Dagdas, K. Krasileva, NLR diversity, helpers and integrated domains: Making sense of the NLR IDentity. *Curr. Opin. Plant Biol.* **38**, 59–67 (2017).

10. B. H. Wang, D. J. Ebbole, Z. H. Wang, The arms race between *Magnaporthe oryzae* and rice: Diversity and interaction of Avr and R genes. *J. Integr. Agric.* **16**, 2746–2760 (2017).

11. E. M. L. Duxbury, J. P. Day, D. Maria Vespasiani, Y. Thüringer, I. Tolosana, S. C. Smith, L. Tagliaferri, A. Kamacioglu, I. Lindsley, L. Love, R. L. Unckless, F. M. Jiggins, B. Longdon, Host-pathogen coevolution increases genetic variation in susceptibility to infection. *eLife* **8**, e46440 (2019).
12. Y. Zhao, J. Huang, Z. Wang, S. Jing, Y. Wang, Y. Ouyang, B. Cai, X.-F. Xin, X. Liu, C. Zhang, Y. Pan, R. Ma, Q. Li, W. Jiang, Y. Zeng, X. Shangguan, H. Wang, B. Du, L. Zhu, X. Xu, Y.-Q. Feng, S. Y. He, R. Chen, Q. Zhang, G. He, Allelic diversity in an NLR gene BPH9 enables rice to combat planthopper variation. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 12850–12855 (2016).

13. F. Gösser, M. Schartl, F. J. García-De León, R. Tollrian, K. P. Lampert, Red Queen revisited: Immune gene diversity and parasite load in the asexual *Poecilia formosa* versus its sexual host species *P. mexicana*. *PLOS ONE* **14**, e0219000 (2019).

14. K. J. Peters, C. Evans, J. D. Aguirre, S. Kleindorfer, Genetic admixture predicts parasite intensity: Evidence for increased hybrid performance in Darwin’s tree finches. *R. Soc. Open Sci.* **6**, 181616 (2019).

15. P. S. White, A. Choi, R. Pandey, A. Menezes, M. Penley, A. K. Gibson, J. de Roode, L. Morran, Host heterogeneity mitigates virulence evolution. *Biol. Lett.* **16**, 20190744 (2020).

16. C. M. Lively, A review of Red Queen models for the persistence of obligate sexual reproduction. *J. Hered.* **101**, S13–S20 (2010).

17. L. T. Morran, O. G. Schmidt, I. A. Gelarden, R. C. Parrish, C. M. Lively, Running with the Red Queen: Host-parasite coevolution selects for biparental sex. *Science* **333**, 216–218 (2011).

18. S. K. J. R. Auld, S. K. Tinkler, M. C. Tinsley, Sex as a strategy against rapidly evolving parasites. *Proc. Biol. Sci.* **283**, 20162226 (2016).

19. J. Jaenike, An hypothesis to account for the maintenance of sex within populations. *Evol. Theory* **3**, 191–194 (1978).

20. G. Bell, J. M. Smith, Short-term selection for recombination among mutually antagonistic species. *Nature* **328**, 66–68 (1987).
21. W. D. Hamilton, R. Axelrod, R. Tanese, Sexual reproduction as an adaptation to resist parasites (a review). *Proc. Natl. Acad. Sci. U.S.A.* **87**, 3566–3573 (1990).

22. A. MacPherson, S. P. Otto, Joint coevolutionary–epidemiological models dampen Red Queen cycles and alter conditions for epidemics. *Theor. Popul. Biol.* **122**, 137–148 (2018).

23. A. K. Gibson, J. Y. Xu, C. M. Lively, Within-population covariation between sexual reproduction and susceptibility to local parasites. *Evolution* **70**, 2049–2060 (2016).

24. M. Tobler, I. Schlupp, Expanding the horizon: The Red Queen and potential alternatives. *Can. J. Zool.* **86**, 765–773 (2008).

25. A. K. Gibson, L. F. Delph, D. Vergara, C. M. Lively, Periodic, parasite-mediated selection for and against sex. *Am. Nat.* **192**, 537–551 (2018).

26. S. P. Otto, S. L. Nuismer, Species interactions and the evolution of sex. *Science* **304**, 1018–1020 (2004).

27. S. P. Otto, The evolutionary enigma of sex. *Am. Nat.* **174**, S1–S14 (2009).

28. J. Engelstädter, S. Bonhoeffer, Red Queen dynamics with non-standard fitness interactions. *PLOS Comput. Biol.* **5**, e1000469 (2009).

29. R. D. Kouyos, M. Salathé, S. P. Otto, S. Bonhoeffer, The role of epistasis on the evolution of recombination in host–parasite coevolution. *Theor. Popul. Biol.* **75**, 1–13 (2009).

30. J. Engelstädter, Host–parasite coevolutionary dynamics with generalized success/failure infection genetics. *Am. Nat.* **185**, E117–E129 (2015).

31. A. Agrawal, C. M. Lively, . *Evol. Ecol. Res.* **4**, 79–90 (2002).

32. P. H. Thrall, L. G. Barrett, P. N. Dodds, J. J. Burdon, Epidemiological and evolutionary outcomes in gene-for-gene and matching allele models. *Front. Plant Sci.* **6**, 1084 (2016).
33. A. Sasaki, Host–parasite coevolution in a multilocus gene-for-gene system. *Proc. Biol. Sci. 267*, 2183–2188 (2000).

34. C.-X. Li, W. A. Cowling, Identification of a single dominant allele for resistance to blackleg in *Brassica napus* ‘Surpass 400’. *Plant Breed. 122*, 485–488 (2003).

35. A. Tellier, J. K. M. Brown, Polymorphism in multilocus host–parasite coevolutionary interactions. *Genetics 177*, 1777–1790 (2007).

36. L. Wilfert, P. Schmid-Hempel, The genetic architecture of susceptibility to parasites. *BMC Evol. Biol. 8*, 187 (2008).

37. C. M. J. A. Metzger, P. Luijckx, G. Bento, M. Mariadassou, D. Ebert, The Red Queen lives: Epistasis between linked resistance loci. *Evolution 70*, 480–487 (2016).

38. A. Templeton, Epistasis and complex traits, in *Epistasis and the Evolutionary Process*, J. Wolf, B. I. Brodie, M. Wade, Eds. (Oxford Univ. Press, 2000).

39. P. Schmid-Hempel, *Evolutionary Parasitology. The Integrated Study of Infections, Immunology, Ecology, and Genetics* (Oxford Univ. Press, ed. 2, 2021).

40. H. H. Flor, Host-parasite interactions in flax rust—Its genetics and other implications. *Phytopathology 45*, 680–685 (1955).

41. G. S. Sidhu, Parasitic epistasis. *Phytopathology 74*, 382–384 (1984).

42. R. Cogni, C. Cao, J. P. Day, C. Bridson, F. M. Jiggins, The genetic architecture of resistance to virus infection in *Drosophila*. *Mol. Ecol. 25*, 5228–5241 (2016).

43. G. Bussotti, L. Piel, P. Pescher, M. Domagalska, S. Rajan, T. Doniger, D.-G. Hiregange, P. Myler, R. Unger, S. Michaeli, G. Spáth, Genome instability drives epistatic adaptation in the human pathogen *Leishmania*. *Proc. Natl. Acad. Sci. U.S.A. 118*, e2113744118 (2021).

44. B. Ashby, S. Gupta, A. Buckling, Effects of epistasis on infectivity range during host–parasite coevolution. *Evolution 68*, 2972–2982 (2014).
45. M. Lynch, H.-W. Deng, Genetic slippage in response to sex. *Am. Nat.* **144**, 242–261 (1994).

46. E. Decaestecker, L. De Meester, J. Mergeay, Cyclical parthenogenesis in *Daphnia*: Sexual versus asexual reproduction, in *Lost Sex*, I. Schön, K. Martens, P. Dijk, Eds. (Springer Netherlands, 2009), pp. 295–316.

47. D. S. Falconer, *Introduction to Quantitative Genetics* (Longmans Green, ed. 2, 1981).

48. L. Becks, A. F. Agrawal, The effect of sex on the mean and variance of fitness in facultatively sexual rotifers. *J. Evol. Biol.* **24**, 656–664 (2011).

49. L. Becks, A. F. Agrawal, The evolution of sex is favoured during adaptation to new environments. *PLOS Biol.* **10**, e1001317 (2012).

50. C. Ameline, Y. Bourgeois, F. Vögtli, E. Savola, J. Andras, J. Engelstädter, D. Ebert, A two-locus system with strong epistasis underlies rapid parasite-mediated evolution of host resistance. *Mol. Biol. Evol.* **38**, 1512–1528 (2021).

51. D. Duneau, P. Luijckx, F. Ben-Ami, C. Laforsch, D. Ebert, Resolving the infection process reveals striking differences in the contribution of environment, genetics and phylogeny to host–parasite interactions. *BMC Biol.* **9**, 11 (2011).

52. M. Fredericksen, C. Ameline, M. Krebs, B. Hüussy, P. D. Fields, J. P. Andras, D. Ebert, Infection phenotypes of a coevolving parasite are highly diverse, structured, and specific. *Evolution* **75**, 2540–2554 (2021).

53. P. Luijckx, H. Fienberg, D. Duneau, D. Ebert, A matching-allele model explains host resistance to parasites. *Curr. Biol.* **23**, 1085–1088 (2013).

54. G. Bento, J. Routtu, P. D. Fields, Y. Bourgeois, L. Du Pasquier, D. Ebert, The genetic basis of resistance and matching-allele interactions of a host-parasite system: The *Daphnia magna-Pasteuria ramosa* model. *PLOS Genet.* **13**, e1006596 (2017).
55. G. Bento, P. D. Fields, D. Duneau, D. Ebert, An alternative route of bacterial infection associated with a novel resistance locus in the *Daphnia–Pasteuria* host–parasite system. *Heredity* **125**, 173–183 (2020).

56. M. A. Duffy, L. Sivars-Becker, Rapid evolution and ecological host–parasite dynamics. *Ecol. Lett.* **10**, 44–53 (2007).

57. A. B. Duncan, T. J. Little, Parasite-driven genetic change in a natural population of *Daphnia*. *Evolution* **61**, 796–803 (2007).

58. E. Decaestecker, S. Gaba, J. A. M. Raeymaekers, R. Stoks, L. Van Kerckhoven, D. Ebert, L. De Meester, Host–parasite ‘Red Queen’ dynamics archived in pond sediment. *Nature* **450**, 870–873 (2007).

59. A. D. Morgan, B. Koskella, Coevolution of host and pathogen, in *Genetics and Evolution of Infectious Diseases*, M. Tibayrenc, Ed. (Elsevier, ed. 2, 2017), pp. 115–140.

60. B. Koskella, Resistance gained, resistance lost: An explanation for host–parasite coexistence. *PLOS Biol.* **16**, e3000013 (2018).

61. A.-L. Laine, Role of coevolution in generating biological diversity: Spatially divergent selection trajectories. *J. Exp. Bot.* **60**, 2957–2970 (2009).

62. E. González-Tortuero, J. Rusek, P. Turko, A. Petrusek, I. Maayan, L. Piálek, C. Tellenbach, S. Gießler, P. Spaak, J. Wolinska, *Daphnia* parasite dynamics across multiple *Caullerya* epidemics indicate selection against common parasite genotypes. *Zool.* **119**, 314–321 (2016).

63. M. F. Dybdahl, C. E. Jenkins, S. L. Nuismer, Identifying the molecular basis of host-parasite coevolution: Merging models and mechanisms. *Am. Nat.* **184**, 1–13 (2014).

64. T. J. Little, H.-J. Carius, O. Sakwinska, D. Ebert, Competitiveness and life-history characteristics of *Daphnia* with respect to susceptibility to a bacterial pathogen. *J. Evol. Biol.* **15**, 796–802 (2002).
65. M. Ślusarczyk, S. Flis, Light quantity, not photoperiod terminates diapause in the crustacean *Daphnia*. *Limnol. Oceanogr.* **64**, 124–130 (2019).

66. L. Orsini, H. Marshall, M. Cuenca Cambronero, A. Chaturvedi, K. W. Thomas, M. E. Pfrender, K. I. Spanier, L. De Meester, Temporal genetic stability in natural populations of the waterflea *Daphnia magna* in response to strong selection pressure. *Mol. Ecol.* **25**, 6024–6038 (2016).

67. R. Chaix, C. Cao, P. Donnelly, Is mate choice in humans MHC-dependent? *PLOS Genet.* **4**, e1000184 (2008).

68. Y. Jiang, D. I. Bolnick, M. Kirkpatrick, Assortative mating in animals. *Am. Nat.* **181**, E125–E138 (2013).

69. M. Salathé, R. Kouyos, S. Bonhoeffer, The state of affairs in the kingdom of the Red Queen. *Trends Ecol. Evol.* **23**, 439–445 (2008).

70. F. Altermatt, D. Ebert, Genetic diversity of *Daphnia magna* populations enhances resistance to parasites. *Ecol. Lett.* **11**, 918–928 (2008).

71. S. D. Desai, R. W. Currie, Genetic diversity within honey bee colonies affects pathogen load and relative virus levels in honey bees, *Apis mellifera* L, *Behav. Ecol. Sociobiol.* **69**, 1527–1541 (2015).

72. A. P. Cabalzar, P. D. Fields, Y. Kato, H. Watanabe, D. Ebert, Parasite-mediated selection in a natural metapopulation of *Daphnia magna*. *Mol. Ecol.* **28**, 4770–4785 (2019).

73. J. M. Broniewski, S. Meaden, S. Paterson, A. Buckling, E. R. Westra, The effect of phage genetic diversity on bacterial resistance evolution. *ISME J.* **14**, 828–836 (2020).

74. S. Sallinen, A. Norberg, H. Susi, A.-L. Laine, Intraspecific host variation plays a key role in virus community assembly. *Nat. Commun.* **11**, 5610 (2020).
75. C. Eizaguirre, T. L. Lenz, M. Kalbe, M. Milinski, Rapid and adaptive evolution of MHC genes under parasite selection in experimental vertebrate populations. *Nat. Commun.* **3**, 621 (2012).

76. P. W. Hedrick, Pathogen resistance and genetic variation at MHC loci. *Evolution* **56**, 1902–1908 (2002).

77. S. K. J. R. Auld, J. Brand, Simulated climate change, epidemic size, and host evolution across host-parasite populations. *Glob. Chang. Biol.* **23**, 5045–5053 (2017).

78. D. Ebert, D. Duneau, M. D. Hall, P. Luijckx, J. P. Andras, L. Du Pasquier, F. Ben-Ami, A population biology perspective on the stepwise infection process of the bacterial pathogen *Pasteuria ramosa* in *Daphnia*. *Adv. Parasitol.* **91**, 265–310 (2016).

79. D. Ebert, Experimental evolution of parasites. *Science* **282**, 1432–1436 (1998).

80. P. Luijckx, F. Ben-Ami, L. Mouton, L. Du Pasquier, D. Ebert, Cloning of the unculturable parasite *Pasteuria ramosa* and its *Daphnia* host reveals extreme genotype–genotype interactions. *Ecol. Lett.* **14**, 125–131 (2011).

81. S. E. Mitchell, E. S. Rogers, T. J. Little, A. F. Read, Host-parasite and genotype-by-environment interactions: Temperature modifies potential for selection by a sterilizing pathogen. *Evolution* **59**, 70–80 (2005).

82. P. F. Vale, M. Stjernman, T. J. Little, Temperature-dependent costs of parasitism and maintenance of polymorphism under genotype-by-environment interactions. *J. Evol. Biol.* **21**, 1418–1427 (2008).

83. T. E. Hector, C. M. Sgrò, M. D. Hall, Pathogen exposure disrupts an organism’s ability to cope with thermal stress. *Glob. Chang. Biol.* **25**, 3893–3905 (2019).

84. P. F. Vale, A. J. Wilson, A. Best, M. Boots, T. J. Little, Epidemiological, evolutionary, and coevolutionary implications of context-dependent parasitism. *Am. Nat.* **177**, 510–521 (2011).
85. P. F. Vale, T. J. Little, Measuring parasite fitness under genetic and thermal variation. *Heredity* **103**, 102–109 (2009).

86. A.-L. Laine, Pathogen fitness components and genotypes differ in their sensitivity to nutrient and temperature variation in a wild plant–pathogen association. *J. Evol. Biol.* **20**, 2371–2378 (2007).

87. M. A. Duffy, K. K. Hunsberger, Infectivity is influenced by parasite spore age and exposure to freezing: Do shallow waters provide *Daphnia* a refuge from some parasites? *J. Plankton Res.* **41**, 12–16 (2019).

88. T. Dallas, J. M. Drake, Fluctuating temperatures alter environmental pathogen transmission in a *Daphnia*-pathogen system. *Ecol. Evol.* **6**, 7931–7938 (2016).

89. D. Kirk, N. Jones, S. Peacock, J. Phillips, P. K. Molnár, M. Krkošek, P. Luijckx, Empirical evidence that metabolic theory describes the temperature dependency of within-host parasite dynamics. *PLOS Biol.* **16**, e2004608 (2018).

90. D. Kirk, P. Luijckx, A. Stanic, M. Krkošek, Predicting the thermal and allometric dependencies of disease transmission via the metabolic theory of ecology. *Am. Nat.* **193**, 661–676 (2019).

91. M. S. Shocket, A. T. Strauss, J. L. Hite, M. Šljivar, D. J. Civitello, M. A. Duffy, C. E. Cáceres, S. R. Hall, Temperature drives epidemics in a zooplankton-fungus disease system: A trait-driven approach points to transmission via host foraging. *Am. Nat.* **191**, 435–451 (2018).

92. M. S. Shocket, D. Vergara, A. J. Sickbert, J. M. Walsman, A. T. Strauss, J. L. Hite, M. A. Duffy, C. E. Cáceres, S. R. Hall, Parasite rearing and infection temperatures jointly influence disease transmission and shape seasonality of epidemics. *Ecology* **99**, 1975–1987 (2018).

93. L. Reyserhove, G. Samaey, K. Muylaert, V. Coppé, W. Van Colen, E. Decaestecker, A historical perspective of nutrient change impact on an infectious disease in *Daphnia*. *Ecology* **98**, 2784–2798 (2017).
94. J. L. Hite, C. E. Cressler, Resource-driven changes to host population stability alter the evolution of virulence and transmission. *Philos. Trans. R. Soc. B Biol. Sci.* **373**, 20170087 (2018).

95. M. P. Sison-Mangus, C. M. J. A. Metzger, D. Ebert, Host genotype-specific microbiota do not influence the susceptibility of *D. magna* to a bacterial pathogen. *Sci. Rep.* **8**, 9407 (2018).

96. M. A. Duffy, S. R. Hall, A. J. Tessier, M. Huebner, Selective predators and their parasitized prey: Are epidemics in zooplankton under top-down control? *Limnol. Oceanogr.* **50**, 412–420 (2005).