EVOLUTIONARY BIOLOGY

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

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Although parasite-mediated selection is a major driver of host evolution, its influence on genetic variation for parasite resistance is not yet well understood. We monitored resistance in a large population of the planktonic crustacean *Daphnia magna* over 8 years, as it underwent yearly epidemics of the bacterial pathogen *Pasteuria ramosa*. We observed cyclic dynamics of resistance: Resistance increased throughout the epidemics, but susceptibility was restored each spring when hosts hatched from sexual resting stages. Host resting stages collected across the year showed that largely resistant host populations can produce susceptible sexual offspring. A genetic model of resistance developed for this host-parasite system, based on multiple loci and strong epistasis, is in partial agreement with our findings. Our results reveal that, despite strong selection for resistance in a natural host population, genetic slippage after sexual reproduction can be a strong factor for the maintenance of genetic diversity of host resistance.

INTRODUCTION

The origin and maintenance of diversity is a major question in evolutionary biology, with the respective roles of selection, mutation, and drift in maintaining genetic diversity in nature still being disputed (*1*–*4*). Parasites, including pathogens, have been suggested as a causal factor for some highly diverse regions in plant and animal genomes. The role of selection by parasites is well established for the major histocompatibility (MHC) gene complex in jawed vertebrates and resistance (R) genes in plants (*5*–*7*), both of which have remarkably high genetic diversity (*8*, *9*). In particular, selection by parasites is linked to increased host diversity (*10*, *11*), and high diversity at resistance genes has been shown to be advantageous against parasites (*8*, *12*–*15*).

As a key mechanism for creating diversity via novel allele combinations, sexual reproduction is a central component of host-parasite coevolution theory (*16*–*18*). Recombination may allow a host population to create new genotypes to which the common parasites are not yet adapted to, thereby reducing the damage caused by parasites adapted to specific host genotypes. On the basis of this reasoning, it has been suggested that parasites select for the maintenance of host sexual reproduction as a mechanism to create and maintain beneficial genetic diversity—the Red Queen hypothesis (*19*–*22*). Parasites have been shown to promote sex and outbreeding (*17*, *23*), and there is empirical evidence of the advantage of sexual over asexual reproduction in natural systems and associated experiments (*18*, *24*, *25*). On the other hand, sexual reproduction may represent a cost for a population that has adapted to its local environment, because it may destroy advantageous allele combinations (*26*, *27*). Models have shown that recombination could be selected against, under certain conditions of genetic interactions and selection (*28*–*30*).

A crucial aspect for our understanding of disease trait evolution is the genetic architecture of the traits under selection, i.e., the number of loci involved, linkage among these loci, dominance, and epistatic interaction among loci. Red Queen dynamics assume specific forms of genetic architecture for resistance, without which polymorphisms at loci under selection would disappear (*26*, *31*, *32*). Epistasis, i.e., nonadditive action of alleles at different loci, and—for diploid organisms—dominance play a particular role in host-parasite interactions because both are central in the effect of recombination on phenotypic variation (*26*, *27*). For most host-parasite systems, however, we know little about the link between the genetic architecture of resistance, the effect of selection, and the role of genetic recombination for the evolution of the system. Empirical and theoretical work determining resistance to parasites in natural systems has suggested a genetic architecture with few loci, with dominance and epistasis, for most systems (*33*–*37*).

Because it is difficult to investigate properly, epistasis is an underrated factor in evolution, but is believed to be very common (*38*). Epistasis in host-parasite interactions is increasingly recognized from diverse host-parasite systems [reviewed in table 10.1, p. 249 in (*39*)]. The gene-for-gene model (GFG), discovered by Flor (*40*) about 70 years ago in the flax-*Melampsora* system, is characterized by its strong nonadditive genetic effects, with epistasis being common in multilocus GFG interaction in many plant-parasite systems [reviewed in (*41*)]. In fewer cases of animal-parasite systems, nonadditive genetic effects were found [reviewed in (*36*)], recent examples including *Drosophila*-virus systems (*42*) and human-*Leishmania* (*43*). Epistasis has also been seen in bacteria-phage interactions [reviewed in (*44*)]. Epistasis can have a major impact on trait expression and therefore may impede or enhance the response to selection (*27*, *45*). Here, we aim to understand the role of epistasis and sexual recombination in shaping mean and variance of parasite resistance and its consequences for the evolutionary dynamics of resistance.

Many organisms in diverse taxa, such as cladocerans, monogonont rotifers, bryozoan, and aphids, reproduce by cyclical parthenogenesis. They produce parthenogenetic offspring directly throughout most of the season, with occasional periods of sexual reproduction that result in resting stages that usually hatch at the beginning of the following season (*46*). In such a reproductive system, selection is

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expected to increase the mean fitness of the population during periods of asexual reproduction. After sexual recombination, the mean fitness of the population is expected to decrease again, a phenomenon known as regression to the mean before selection (*47*), or genetic slippage in response to sex (*45*, *46*). This phenomenon occurs because the most extreme phenotypes are based on genotypes that represent allele combinations that are particular in their specific combination so that breaking up these combinations will change the phenotype away from the extreme and thus move the average phenotype of the offspring back toward the mean of the population before selection. The effect hinges on the nonadditivity of the alleles, i.e., on the presence of dominance and/or epistasis (*45*–*47*). The variance of the trait under selection is also expected to change, although the direction of the change cannot be easily predicted as it depends on the signs of the covariances between genetic effects in the parental generation (*45*, *46*). In rotifer populations, variance has been observed to both increase and decrease after sexual reproduction (*48*, *49*). Because of their extended period of asexual reproduction, cyclic parthenogens are good systems to study genetic slippage (*48*). During the asexual phase, selection over time can build up high frequencies of the most favored genotype (*47*), making the effect of both selection and recombination on the trait under selection more evident.

We monitored resistance phenotypic changes over eight consecutive years in a large natural population of the crustacean *Daphnia magna*, whose yearly population cycle includes strong summer epidemics of the bacterial parasite *Pasteuria ramosa*, sexual reproduction to survive the winter, and the hatching of sexual offspring in spring. In a previous study, we documented parasite-mediated selection in this population and resolved parts of the underlying genetic architecture of resistance to the local parasite (*50*). Here, we show that resistance increases during the yearly parasite epidemics, but that sexual recombination reestablishes the initial resistance diversity seen among the sexual offspring hatching in the following planktonic season. We thus reveal an extreme case of genetic slippage created by sexual reproduction in this cyclical parthenogenetic host. We link this long-term monitoring to a system-specific genetic model of resistance that predicts the impact of sexual reproduction on the temporal dynamics of the evolution of resistance. This model includes dominance and epistasis at resistance loci, stressing their role in genetic slippage and, thus, their contribution to explaining the maintenance of genetic diversity for resistance.

RESULTS

Seasonal epidemics

We monitored a large *D. magna* population in the fishless Lake Aegelsee, Switzerland (*50*) from 9 October 2010 to 24 September 2018, observing strong annual epidemics of *P. ramosa* that typically started in early May, about a month after the host emerged from diapause and lasted through most of the summer (Fig. 1A). Epidemics reached peak prevalence of 70% to nearly 100%; no epidemic of any other known *D. magna* parasite was observed in this population. The population overwinters exclusively in the form of sexually produced resting stages, with an estimated overwintering population size of several millions. Earlier breeding experiments confirmed that resting stage production is entirely sexual (*50*). Thus, to the best of our knowledge, no genotypes (clones) survive from 1 year to the next. We also monitored environmental and ecological variables over the course of our study and present those results in fig. S1 and text S1.

Resistotype dynamics

Using five isolates of the pathogen *P. ramosa*, we quantified the proportion of resistant (R) and susceptible (S) host phenotypes in the population with an attachment test that measures the parasite's ability to attach to the host cuticle; failure to attach indicates resistant hosts (*51*). Because we can clone females using the host's parthenogenetic eggs (iso-female lines), we can perform this test on several individuals with the same genotype. Resistotypes—i.e., resistance phenotypes—are here presented as a sequence of R and S letters, each letter representing resistance or susceptibility to one of the five tested parasite isolates in the following order: C1, C19, P15, P20, and P21. We used the placeholder "␣" for isolates that we did not test or consider. P20 and P21 were isolated from our local population, while C1, C19, and P15 come from other European populations and were used previously, along with the local P20, to build the genetic model for resistance in the host (*50*). The nonnative parasite isolates are, however, representatives of the larger diversity of the parasite that is also present in the population studied here (*52*).

For eight successive years, we observed similar resistotype frequencies in spring (Fig. 1B). From 2011 to 2013, when data resolution was lower because of less frequent sampling and smaller sample sizes, the spring cohort was composed of about 25% of the SS␣␣␣ resistotype and 75% of the RR␣␣␣ resistotype (Fig. 1B). Two additional *P. ramosa* isolates were added from 2014 and one more from 2016 onward, allowing for a more refined picture that was dominated by four phenotypes: Resistotypes SSSS␣ and RRSS␣ each represented about 25% of the population, RRSR␣ represented about 45%, and RRRR␣ represented about 5% (Fig. 1B). Overall, R resistotypes were more common for C1, C19, and P20, while S resistotypes were more common for the P15 and P21 parasites (Fig. 1C).

Each year, these resistotype frequencies were relatively stable at the beginning of the season but changed markedly after the start of the *P. ramosa* epidemic in May. Two resistant phenotypes, namely, RRSR␣ and RRRR␣ (blue in Fig. 1B, 2014–2018), increased in proportion, while the resistotypes susceptible to P15 and P20—RRSS␣ and SSSS␣—decreased in proportion (orange and yellow in Fig. 1B). Overall, resistance to all individual *P. ramosa* strains increased over the season (dark gray in Fig. 1C): Resistance to C1 and C19 increased every year from 79 ± 2% to 97 ± 1% during the entire 6 months of the *D. magna* planktonic phase. The biggest change was resistance to P20, which increased from 49 ± 4% to 96 ± 2% within 2 months during the main peak of the epidemics. Resistance to P15 and P21 showed a more complex pattern, with a tendency to increase during the second half of the summer and decrease again toward the end of the season (Fig. 1C).

The stable spring frequencies across years, together with the strong dynamics across the summer season, resulted in a strong pattern of cyclic resistotype frequencies changes. Among about 4000 tested genotypes across 8 years, some resistotypes were never observed in our samples, e.g., SS␣R␣ and RS␣␣␣, indicating genetically impossible phenotype combinations or absence of polymorphisms at the underlying resistance loci in this population (*50*).

Response to selection for resistance

As a cyclic parthenogen, *D. magna* reproduces asexually during most of the active season and produces sexual resting stages in a protective case (ephippium) that overwinter and hatch in the spring. Because the planktonic animals do not overwinter in our population, the spring cohort is exclusively the result of sexual reproduction.

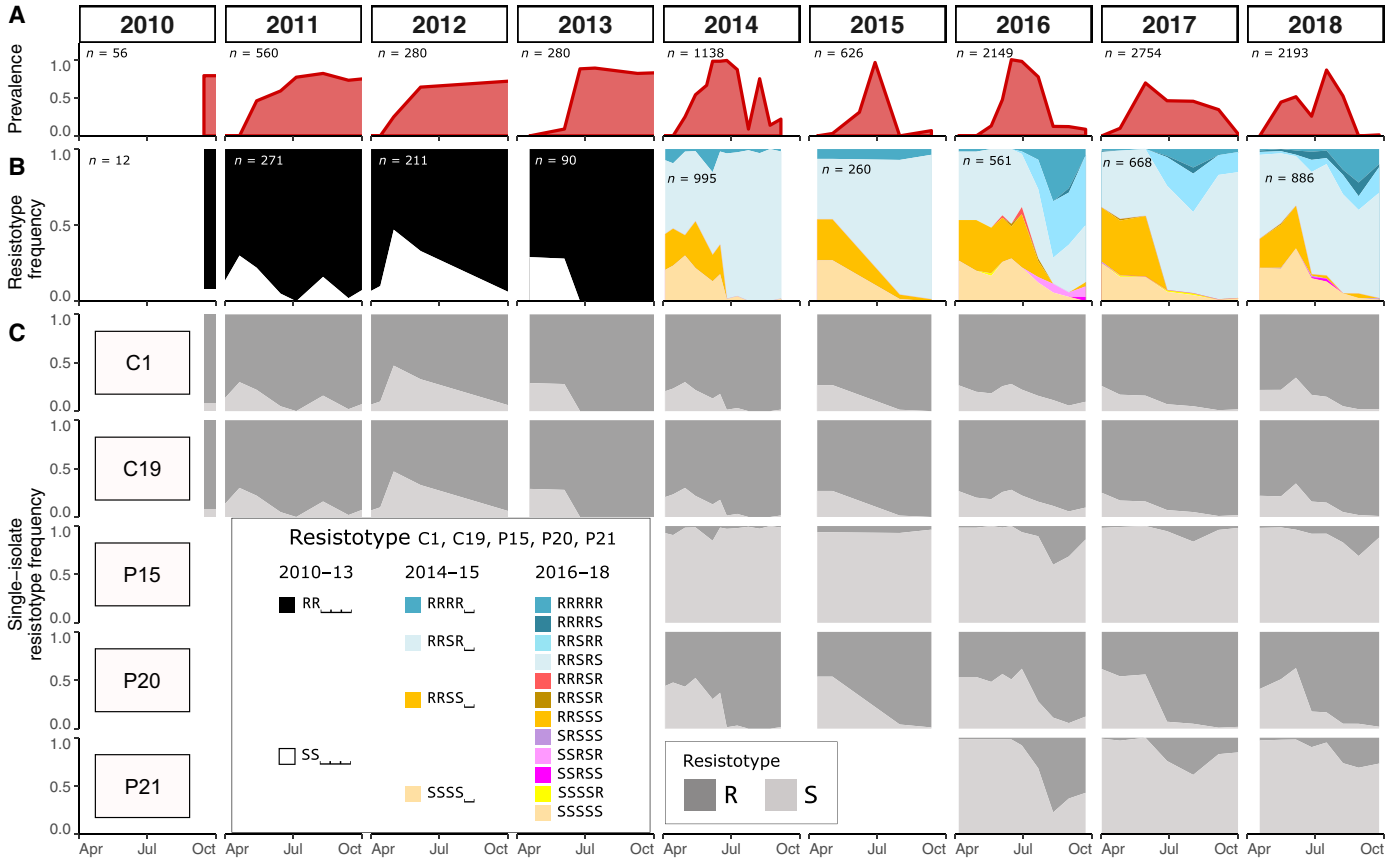

Fig. 1. Cyclic resistotype dynamics across 8 years in the Aegelsee. From 2010 to 2018, samples of *D. magna* were collected from early April to early October every 2 to 4 weeks. Parasite prevalence was recorded, and about 60 to 100 animals were cloned and their resistotypes (resistance phenotypes) were assessed. (**A**) *P. ramosa* prevalence (=proportion of infected females) in the *D. magna* population. (**B**) Resistotype frequency in the *D. magna* population. Resistance and susceptibility to individual *P. ramosa* isolates are denoted as R and S, respectively. The combined resistotype shows resistance for up to five *P. ramosa* isolates: C1, C19, P15, P20, and P21. Until 2013, only C1 and C19 were tested; in 2014 and 2015, isolates C1, C19, P15, and P20 were tested; and all five isolates were tested after 2015. We use the placeholder _, when an isolate was not tested. Resistance to P20 is pinpointed because of its importance in the evolution of the host population (*50*). *n* denotes the total number of genotypes tested in a given year. (**C**) Resistotype frequency to each of the five *P. ramosa* isolates. Note the strong increase in resistance to P20 every year.

To look at the impact of selection and recombination on resistance diversity during and between seasons, respectively, we calculated the mean and variance of resistance phenotypes of the planktonic population for each sample through time, assigning resistance (R) and susceptibility (S) a value of 1 and 0, respectively. If directional selection acts on resistance, we expect mean resistance to increase, as selection removes susceptible phenotypes. As hardly any susceptible resistotypes are left at the end of the summer, we further expected variance in resistance to decrease during the summer, as resistance reaches high values. Furthermore, a round of sexual reproduction is expected to restore, or partly restore, the variance, and the mean is expected to relapse to some degree because genetic recombination leads (under most conditions) to a regress to the mean before selection (*47*), also discussed as genetic slippage (*45*, *46*). Our results align with these predictions: Every year, mean resistance increased and variance declined over the planktonic season (Fig. 2). After sexual reproduction, variance was restored, and the mean regressed toward the mean of the previous year before selection. What was surprising, however, was that the relapse of the mean was nearly perfect over the entire study period, showing that there was no overall response to selection across seasons. Note that the apparent drop in the mean between 2015 and 2016 in Fig. 2 is caused by the addition of one more *P. ramosa* isolate (P21) in the test panel.

Selection and sexual reproduction

To understand these pronounced dynamics in mean resistance and its variance, we collected and hatched sexually produced resting stages across three seasons, using sediment traps that we emptied at about monthly intervals. Sediment traps allow us to decouple the current resting stage production from resting stages produced earlier (forming a seed bank–like reservoir), as these traps only collect resting stages that are dropped from the current planktonic population. This allowed us to estimate when sexual reproduction occurred and—by subsequent hatching of resting stages from each sampling date—to estimate the hatchling resistotype frequencies.

We observed that resting stages were produced throughout most of *D. magna*'s planktonic phase and tended to show multiple peaks before and after the main change in resistotype frequencies in June to July (Fig. 3B). The number of resting stages per ephippium (zero, one, or two) produced over the planktonic phase of *D. magna* remained approximately stable (linear regression, all years pooled: $R^2 = 0.14$, $F = 3.2$ on 1 and 13 df, $P = 0.095$; fig. S2, B and C). After

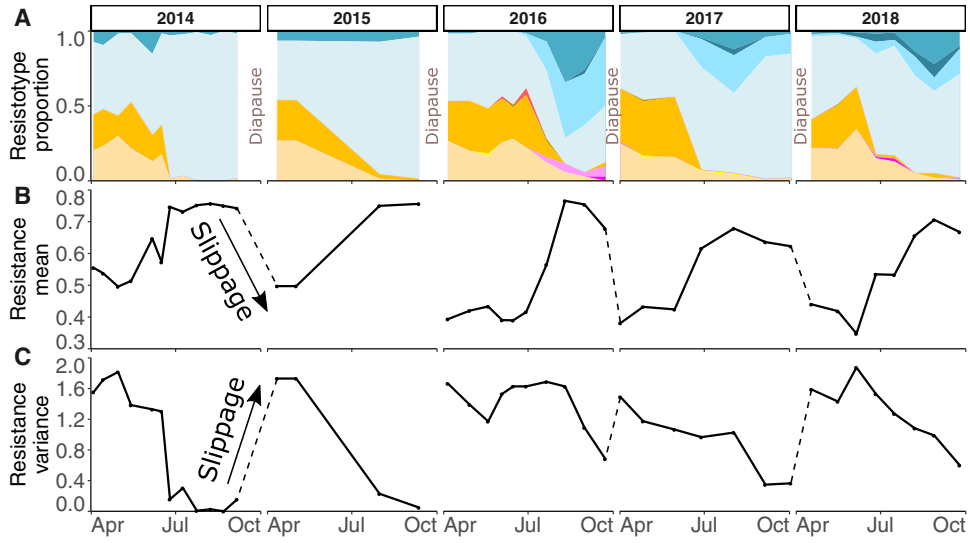

Fig. 2. Genetic slippage resulting from sexual reproduction in the Aegelsee *D. magna* population. (**A**) Observed resistotype (resistance phenotype) frequencies in the *D. magna* population from 2014 to 2018 (same as Fig. 1B for 2014–2018; repeated here for better comparison). (**B**) Mean resistance to *P. ramosa* across time. Mean resistance increases across every summer planktonic phase. We attributed to each resistotype a resistance score ranging from zero to the number of isolates tested, and weighted the mean per sampling point by the number of tested isolates, resulting in a score between zero and one (e.g., RRRRR would have an overall resistance score of 1 and SSSSS would be 0). The dashed lines span the time windows during which sexual offspring overwinter and hatch the following spring. (**C**) Variance of resistance across time, calculated along with the mean in (B). Note that in 2014 and 2015, four bacterial isolates were tested, while we used five from 2016 to 2018; hence, we do not represent the dashed line between 2015 and 2016. Therefore, mean and variance cannot be directly compared between years when different numbers of parasite isolates are used.

diapausing the resting stages in the dark at 4°C, the overall hatching success in outdoor containers in the following spring was 74.4 ± 3.9%, which was independent of the date when the resting stages were collected (linear regression, pooled for all years: R^2 = 0.042, F = 1.75 on 1 and 16 df, P = 0.20). The hatching pattern after induction was also consistent, with most resting stages hatching within a few days after induction (fig. S2). The few resting stages that hatched later did not differ in their resistotype proportions from the earlier hatchlings (measured only in 2014; Fisher's test, P = 0.32; fig. S3).

All hatchlings were cloned and tested for resistotypes. Unexpectedly, in all years, the observed resistotype frequencies of the hatchlings remained rather stable over the season, both for the combined and for the individual bacterial isolates (Fig. 3, C and D), independent of the strongly changing resistotype composition of the planktonic animals at the time of resting stage production (Fig. 3A). This created a substantial difference between the resistotype distribution of the parent population and their sexual offspring, especially in late summer, when we observed that susceptible offspring resistotypes (RRSS_ and SSSS_, orange and yellow in Fig. 3C) were created from a parental population that consisted almost solely of resistant resistotypes (RRSR_ and RRRR_, blue in Fig. 3A). The most resistant resistotypes in the planktonic population were hardly seen in the offspring populations [RRRR_ in 2014 and 2015 (dark blue) and RRRRR, RRRRS, and RRSRR in 2017 (dark and bright blue)] (Fig. 3, A and C).

Resting stages produced during the planktonic phase accumulate over the planktonic season, overwinter, and hatch in the following spring. Pooling the resistotype data of the hatchlings from the sediment traps across the entire season and weighting resistotype frequencies by the abundance of resting stages in each sample is therefore a predictor of the expected resistotype composition for the following spring cohort. These predictions match the resistotype composition of the planktonic population in spring very well for all 3 years (Fig. 4 and figs. S3 and S4), indicating that the populations of hatchlings from the cumulative sediment trap samples are representative of the hatchling cohort in the following spring.

Calculation of expected resistotype frequencies in resting stages

From previous genetic studies, we know that dominance and epistasis are defining features of the inheritance of resistance to *P. ramosa* (*37*, *50*, *53*–*55*). To predict the role of sexual recombination in shaping resistance dynamics, we used an existing genetic model for resistance in our study population to calculate the expected resistotype frequencies in the offspring population at the time of resting stage production (sexual reproduction). These calculations require knowledge of allele frequencies at the resistance loci, which are unknown, but which we estimated using known resistotype distributions and assumptions. Although the published genetic model for resistance includes six loci (A to F), here, we considered variation only at the B, C, D, and E loci (fig. S5). The A and F loci, known from other *D. magna* populations, seem to be monomorphic in the Aegelsee population. Alleles B and d are expected to be rare: Resistotypes determined by the "B-" and "dd" genotypes, regardless of the genotype at other loci, were only observed rarely (Fig. 1) (*50*). Allele frequency at the C and E loci has been previously determined in a spring sample from the Aegelsee *D. magna* population (*50*). Using these C and E loci allele frequencies within each resistotype and fixing the B and D loci to be "bbDD" genotype, we found that expected and observed resistotype frequencies match better than do several other scenarios, e.g., equally distributed allele frequency at the C and E loci (Fig. 3, C and E; figs. S6 and S7; and tables S1 and S2). Expected

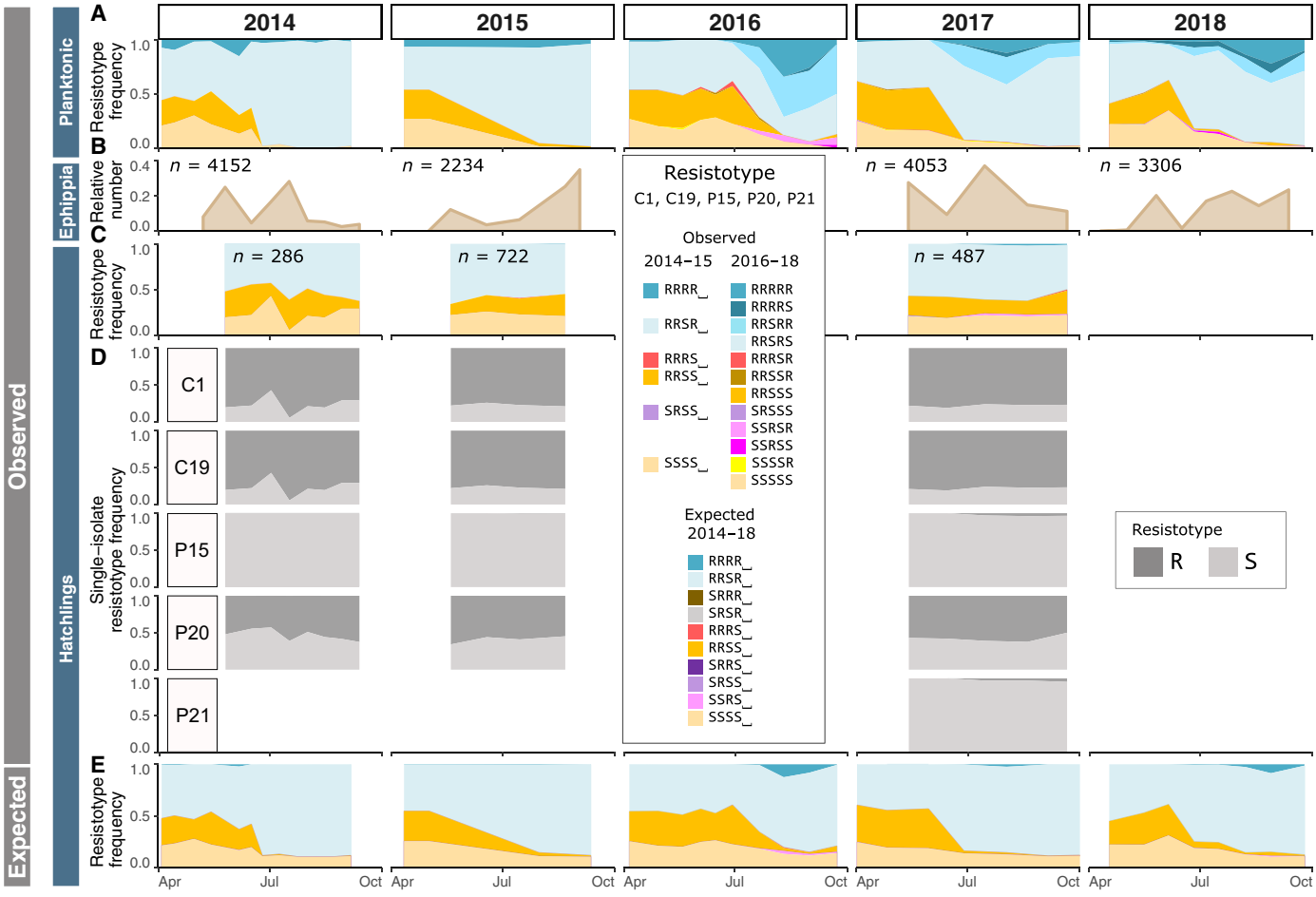

Fig. 3. Longitudinal resting stage hatching of *D. magna* from the Aegelsee. (**A**) Observed resistotype (resistance phenotype) frequencies in the *D. magna* population from 2014 to 2018 (same as Fig. 1B; repeated here for better comparison). (**B**) Observed relative number of *D. magna* resting stage cases (ephippia) produced in the pond and recovered from five to nine sediment traps, in 2- to 4-week intervals from early April to early October in 2014, 2015, 2017, and 2018. Time on the *x* axis represents the midpoint between two consecutive emptying of the traps. *n* indicates the total number of ephippia for a given year. (**C**) Resistotype frequencies of the hatchlings from the sediment traps plotted against the collection time (only for 2014, 2015, and 2017). Resting stages from 2018 were collected but not hatched. Note that in 2014, the first resting stage sample was lost. In 2015, no hatchlings emerged from the last sample. We represent the four-letter resistotype (C1, C19, P15, and P20) to be comparable with (E). (**D**) As in (C) but for each of the five *P. ramosa* isolates separately. (**E**) Expected resistotype frequencies of hatchlings from sexually produced eggs (resting stages) by the planktonic population across the entire planktonic season (also for parts of the season where no resting stages were produced). Expected resistotype frequencies were calculated using the genetic model of resistance in the *D. magna*–*P. ramosa* system, assuming random mating of the parent population at the time of resting stage production. Detailed methods and results of these calculations are given in the text and in figs. S5 and S6 and tables S1 and S2.

and observed resistotype frequencies in the hatchling population match especially well in the first half of the season (Fig. 3, C and E). In the second half of the season, however, we see a marked difference, with the presence of the abundant RRSS_ resistotype (about 25%) not predicted by the model (orange in Fig. 3, C and E).

This discrepancy between predicted and observed resistotype frequencies in the second half of the season may have been due to a nonrepresentative distribution of animals producing the sexual eggs (resting stages) at this time of the year. We tested this by collecting, in August 2020, *D. magna* samples and quantifying the resistotype distribution of females carrying resting stages, of males, and of a random sample of females. We found good correspondence between the random population samples, the sexual females, and the males (fig. S8), indicating that the animals reproducing sexually are a representative sample of the population with regard to resistotypes. In addition, we quantified the resistotype distribution of a random sample of females in April 2021 and found a similar distribution than in the previous spring samples (fig. S8), indicating that the strong genetic slippage has been shaping the resistance profile of this population for 10 years.

DISCUSSION

Genetic variation for parasite resistance in natural populations is observed to be high, but the mechanisms maintaining this variation are not well understood. Here, we address this topic by monitoring the long-term impact of seasonal epidemics of a bacterial pathogen (*P. ramosa*) on the genetic variation in parasite resistance in a natural zooplankton population (*D. magna*). We observed an increase in resistance every summer, coinciding with the parasite epidemics, which have been shown to be driven by parasite-mediated selection on two well-defined genomic regions (*50*). Unexpectedly, despite

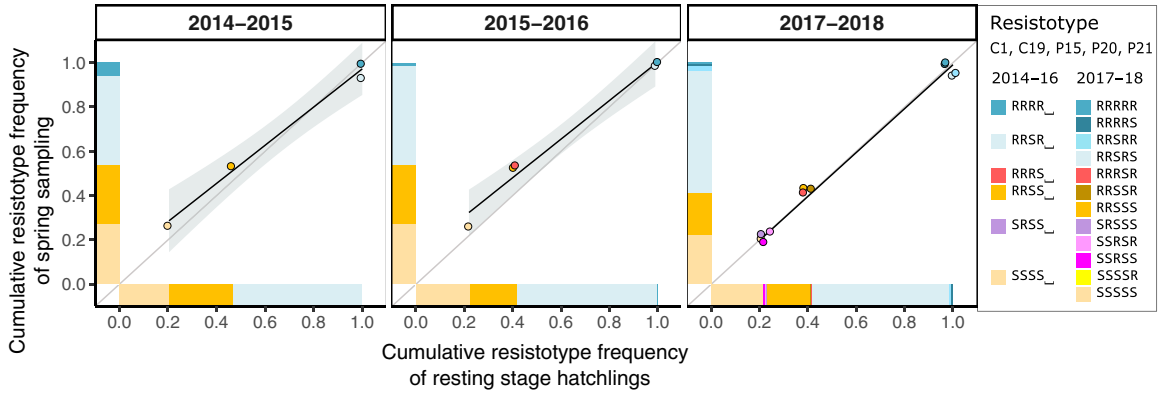

Fig. 4. Scatterplot of resistotype frequencies of the hatchlings from the overwintering resting stages (collected in the sediment traps) against those of the *D. magna* collected the following spring in the Aegelsee. The *x* axis represents cumulated resistotype frequencies in the hatchlings from the sediment traps. These frequencies were calculated by weighing resistotype frequencies in the hatchling population by the relative number of resting stages produced at each sampling point. The *y* axis represents cumulated resistotype frequencies in the first sample collected the following spring after the resting stages. Dots are plotted using jitter to reduce overlap. The gray line represents the $y = x$ function and depicts an expected perfect match between both resistotype frequencies. The black line represents the fitted linear regression, with 95% error as the gray area (not visible in the 2017–2018 panel because it is too small).

the strong selection against susceptible hosts, the sexually produced hatchling population in the following spring showed again high frequencies of susceptible host resistotypes. These spring frequencies remained stable over the 8-year observation period, indicating an apparent absence of a long-term response to selection. These cycles maintain genetic variation for resistance in the host population. We show that the resistance cycles are mostly shaped by the seasonality in production and hatching of sexual resting stages, in combination with the underlying genetics for the inheritance of resistance involving dominance and epistasis. However, our genetic model does not capture the full complexity of the observed dynamics, likely because of an underrepresentation of the isolates used from the local parasite population.

Repeated strong parasite-mediated selection in a natural population

Using materials collected in the Aegelsee in 2014 and 2015, we previously confirmed experimentally that the observed resistotype frequency changes resulted from parasite-mediated selection (*50*). Parasite-mediate selection has been shown to rapidly raise the frequency of resistance in *Daphnia* (*56*–*58*) and in other host-parasite systems [see (*39*, *59*, *60*) for recent reviews], although long-term monitoring of natural populations remains scarce (*25*, *61*, *62*), with notable exceptions in the New Zealand mud snail–trematode system (*23*), a plant-pathogen metapopulation system (*61*), and a chytrid fungus in the *Daphnia longispina* group (*62*).

We observed that the increase in resistance occurred in the host population with some temporal variation in magnitude and speed (see seasonal increase in dark gray in Fig. 1C). Most notably, over five consecutive years, resistance to the sympatric *P. ramosa* isolate P20, which has been shown to play a major role in epidemics in our population (*50*), increased from about 50 to 100% each year within 2 months around the peak of the epidemics. Resistance to *P. ramosa* isolates C1 and C19 consistently increased throughout the planktonic phase each year (from about 75% to nearly 100%), showing that resistance to these infectotypes may also be selected for in the host population. Because these isolates do not come from our focal population, we did not necessarily expect selection for resistance to their infectotypes in the host population. Resistance to these foreign isolates is, however, epistatically linked to resistance to the local isolate P20, as resistance to P20 is only possible when there is resistance to C1 and C19 (*50*). Selection for resistance to the local P20 may then indirectly increase resistance to C1 and C19 rather than direct selection for resistance to these foreign isolates. Furthermore, other yet undescribed parasite lines from our study population may be similar in their host spectrum as these nonnative isolates (*52*). From 2016 to 2018, resistance to P15 (foreign) and P21 (local) increased as well, but decreased somewhat when parasite prevalence declined toward the end of the season. The decline of P15 and P21 susceptibility might be explained by a cost of resistance, as resistant genotypes lose their selective advantage once parasite prevalence declines below some level, allowing susceptible genotypes to increase in frequency. A cost of resistance is expected to occur, regardless of the underlying mechanisms of resistance and infection (*63*). However, no cost of resistance was previously found in this system (*64*). We can exclude genetic drift as an explanation for these cyclic changes, because the *D. magna* census population size in the Aegelsee is estimated at over 10 million individuals with an overwintering resting egg bank of about the same size. In summary, we observed a highly repeatable increase in resistance to the five tested parasite infectotypes every year. This increase in resistance to the local parasite isolates is most likely adaptive, as we showed for P20 (*50*), while the increase in resistance to nonlocal parasites could be a consequence of the genetic architecture of resistance. Moreover, the local parasite population contains distinct genotypes that were not tested in this study [(*52*) and figure S1 in (*50*)]. Despite this increase in resistance, however, susceptibility to the parasite was created anew by a round of sexual reproduction, resulting in a stable long-term genetic diversity for resistance across years (Fig. 1B).

The effects of genetic recombination on resistotype composition

While we cannot exclude gene flow happening among years, we are confident that it did not influence our results because (i) the populations of hatchlings from the cumulative sediment trap samples are representative of these spring cohorts (Fig. 4) and (ii) the sedimentation

rate in the pond is high (1 cm/year), most probably impeding a seedbank effect, as resting stages laid in the previous years would not be able to hatch because of the lack of light and oxygen (*65*).

Decrease of population mean resistance

Because sexual reproduction is a prerequisite for resting stage formation in *D. magna*, we could decouple the effects of selection and genetic recombination on resistance in our host population. Overwintering happens only in the form of sexually produced resting stages, as planktonic individuals die off in early October because of the artificial heating of this sewage pond (fig. S1). Every spring, we observed that the mean resistance of the hatchlings was much below the mean resistance from the previous fall (Fig. 2B). The observation that populations regress back to the mean of the parental population before selection is well known (*27*, *45*, *47*). Prolonged periods of asexual reproduction amplify this effect (*45*). However, despite regression back to the mean of the parental generation, it is usually expected that the offspring mean will move away from the parents, i.e., long-term response to selection. This did not happen in our population, or it is so weak that we did not pick up the signal over the 8-year study period. We suggest that the combination of timing of sexual reproduction and genetic architecture of resistance to isolates that play a role during the epidemics causes these cycles in mean resistance.

We found that resting stages are not only produced at the end of the planktonic season but also already starting in somewhat irregular patterns during the season (Fig. 3B). Resting stages produced at different times did not vary in fitness-related aspects (hatching rate, resting stages per ephippium, and hatching time) (fig. S2), suggesting that their contribution to the next year spring cohort is approximately even. Thus, some of the resting stages that hatched in the spring were produced before selection acted on the parental generation, dampening the overall effect of selection on the spring cohort the following year. However, as typically more than 50% of the resting stages were produced after selection had increased resistance, this alone cannot explain the strong regression to mean resistance.

Genetic recombination reestablishes resistance diversity

We phenotyped the hatchlings of the sexually produced resting stages collected in the sediment traps throughout the season. Early in the season, sexual offspring present approximately the same resistotype distribution as their planktonic parent population. Strikingly, however, resting stages collected late in the season show a markedly different resistotype from the planktonic host population at this time of the year. Namely, the parent population in the late season is composed of mainly resistant animals but produces about 50% susceptible offspring (Fig. 3). Genetic recombination, coupled with a genetic architecture with epistasis and dominance, could create susceptible genotypes out of resistant ones.

To investigate how resistotype diversity is reestablished through sexual reproduction, or how resistant phenotypes can produce susceptible ones, we used a previously published genetic model for the inheritance of resistance in *D. magna* against *P. ramosa* infections (described in fig. S5) (*37*, *50*, *55*). This model allowed us to predict the resistotype frequencies of sexual offspring given a pool of parent resistotypes and their underlying genotypes. We then compared these predicted resistotype frequencies to those we observed among the resting stage hatchlings we collected throughout the season. In the early half of the season, our model worked rather well, with a slight discrepancy between the proportions of the RRSR$_{__}$ resistotype (the model predicted more RRSR$_{__}$ resistotype than observed; light blue in Fig. 3, C and E) and RRSS$_{__}$ (the model predicted less RRSS$_{__}$ than observed; orange in Fig. 3, C and E). Later in the season, we observed a stronger discrepancy between expected and observed resistotype distributions: P20-susceptible resistotypes (RRSS$_{__}$; orange in Fig. 3, C and E) are very common (about 25%) among the sexual offspring resistotypes, although according to our model, they should not be produced by a parent population where P20-resistant resistotypes dominate, because resistance to P20 is recessive (Fig. 3 and fig. S5). The genetic model of resistance displays strong epistasis and dominance, also influencing resistance to P20 (*50*, *55*). Two loci, the C and B loci, epistatically influence resistance to P20, but in the present case, this cannot explain the emergence of RRSS$_{__}$ offspring from a parent population lacking RRSS$_{__}$ individuals. Nevertheless, we believe that, in this multilocus system, epistasis and dominance are the main contributors to the maintenance of genetic diversity for resistance. Our genetic model seems to miss further epistatic interactions between the known loci or additional unknown loci. With multiple loci, dominance, and epistasis, it is difficult to interpret the outcome of genetic crosses, because the number of possibilities increases rapidly.

Our genetic model alone does not allow us to predict the frequencies of resistotypes after recombination without making assumptions about allele frequencies at these loci. We assumed allele frequencies derived from the overall observed resistotype diversity in the population and from previous estimates using genetic markers [see Materials and Methods and (*50*)]. We also assumed that allele frequencies underlying each resistotype did not change across the planktonic phase because we have no reason to expect changes in the frequencies of genotypes coding for the same resistotype. With more knowledge about the actual loci underlying the resistotypes, we may be able to predict resistotype frequencies better in the future. However, changing the assumptions for the allele frequencies did not produce enough of an effect to explain the apparent mismatch between the parent generation and their sexual offspring. In the following section, we explore alternative hypotheses that could explain the discrepancy between the observed and expected resistotype frequencies after recombination.

No evidence for prehatching or prezygotic selection related to resistotype

Prezygotic and/or prehatching selection could also contribute to the observed discrepancies between parent and offspring resistotypes. This could occur if different resistotypes in the planktonic population contributed unequally to sexual reproduction, producing males or resting stages differentially, or copulating at different rates. However, Orsini *et al.* (*66*) suggested that the produced resting stages in *D. magna* populations accurately represent the planktonic population, which agrees with an assessment in our study population indicating that the males and females that participate in sexual reproduction represent the resistotype distribution of the entire population well (fig. S8).

Another form of prezygotic selection could result from negative assortative mating that favors rare susceptible resistotypes. We cannot rule out that nonrandom mating between male and female genotypes or phenotypes (=assortative mating) contributes to the resistotype distribution in the offspring population. Positive assortative mating linked to body size and other traits has been found in a variety of

animals, while negative assortative mating linked to immune genes (MHC) has been found in mice and humans (*67*, *68*). However, assortative mating in relation to immunity or resistance remains to be investigated in invertebrates, and as most population genetics models—including the present study—assume random mating, this is an important aspect for further study. Resistotype-dependent selection during diapause or hatching could also distort resistotype frequencies.

Last, one may speculate that the ephippia we collected from the sediment traps late in the season contained resting stages that had been produced earlier in the season and were resuspended in the water column. However, several arguments speak against this. First, the pond does not contain fish, which may cause bioturbation. Second, the lake has no inflow, but only a very slow outflow, causing no detectable water movement. Third, at times when the *D. Magna* population does not produce resting stages (the spring cohort in April), we find no resting stages in the sediment traps. Fourth, the same redistribution (in quantity and quality) would have needed to occur every year, as we observed the same patterns over 3 years. We thus conclude that water turbation is an unlikely explanation for the observed mismatch between resistotype distributions in the fall planktonic phase and the sexual stages it produced.

The Red Queen theory for the maintenance of sex

Genetic recombination creates novel genotypes and phenotypes on which selection not only can act but also may destroy coadapted gene complexes. At first sight, the latter seems to be the case in our study population because the recombinant offspring are more susceptible than their parents. This seems to go against the idea of the Red Queen hypothesis that genetic recombination is adaptive and therefore may be responsible for the maintenance of sex (*19*, *21*, *26*, *69*). Under this hypothesis, genetic recombination is advantageous for hosts because it can recreate genotypes and phenotypes that were selected out before (*16*, *25*). This does happen in the here studied population, but the recreated genotypes code for phenotypes that are on average more susceptible to the parasites in our test panel. Resistance loci in the *D. magna* system have been shown to interact in different ways with different *P. ramosa* isolates, including a matching genotype model (*53*). It may therefore well be that, in our study population, susceptibility to some parasite isolates goes hand in hand with resistance to other, yet to be characterized, *P. ramosa* genotypes. In our study population, we have currently limited information about the true diversity of *P. ramosa* infectotypes and their frequency dynamic throughout the year. We observed that parasite prevalence was still high after the P20-S hosts had become very rare and presumably also the parasites of the P20 type. We speculate that parasites of the P21 type then become more common, which is consistent with the decline in P21-susceptible hosts late in the season. We know that other parasite infectotypes are present in the population (*50*, *52*) but have no clear picture about their functional role. With better knowledge of the parasite population, we may be able to track yearly shifts in the frequency of parasite infectotypes and relate it to the changing host resistotype frequencies. In this scenario, genetic recombination in the host in the second half of the season may alter the host resistotypes that become the target of late-season parasites.

In this study, we demonstrate strong parasite-mediated selection in a natural host population and elucidate the role of sexual reproduction for diversity in resistance phenotypes. Our work stresses (i) the cyclical nature of host-parasite interactions, (ii) the very fast pace of parasite-driven changes in the host population, and (iii) the fact that sexual recombination plays an important role in reshuffling allele combinations. Because of dominance and epistasis in the genetic architecture of resistance—and potentially further complexity that we have not unveiled yet—genetic reshuffling resets the clock to the time before selection acted, rendering the response to selection zero. Although this is an extreme case of genetic slippage in response to sex, it is a powerful agent to maintain genetic diversity, which is a hallmark of resistance in natural populations of *Daphnia* and other taxa including humans, fish, nematodes, insects, bacteria, and plants (*12*, *15*, *70*–*76*). Genetic slippage has never been described in a natural population before, but we expect it to be common. We discuss the specificity of our system in more detail in text S2. As annual cyclic parthenogenetic species are powerful models to observe selection and slippage and they are common, we invite further studies to explore the generality of genetic slippage. As climatic seasonality seems to determine the dynamics of parasite resistance in our host population, and given the known impact of climate change on epidemics in the *D. magna*–*P. ramosa* system (*77*), we speculate that the dynamics in our study population may change in response to the predicted changes in climatic conditions and seasonality.

For the maintenance of the genetic variation in resistance, we argue that the genetic architecture underlying resistance in our study population is sufficient. Even so, we still lack a complete picture, with further parasite genotypes and possibly further resistance loci remaining to be discovered. The strong cycles observed every year are caused by the combination of strong parasite-mediated selection, the specific genetic architecture for parasite resistance, and the synchronous spring hatching of sexual eggs produced in the previous season.

MATERIALS AND METHODS

The *D. magna*–*P. ramosa* system

D. magna Straus (Cladocera) is a freshwater planktonic crustacean that reproduces by cyclical parthenogenesis. Asexual females produce genetically identical (clones) diploid daughters or sons throughout the season. These females may switch to become sexual, and their haploid eggs need fertilization by males. Sexual eggs, which we call resting stages (precisely: embryos in developmental arrest), are produced, singly or in pairs, in a protective case (=ephippium) and require a resting period before hatching. All hatchlings from resting stages are asexually reproducing females. *Daphnia* filter-feed on planktonic algae and from the sediment surface, which is also how they ingest the transmission stages (=spores) of the bacterial parasite *P. ramosa* (Firmicutes: Bacillales). When infected by *P. ramosa, D. magna* take on a reddish coloration and increase in size (gigantism). Infection results in castration, reducing host reproductive success by 80 to 90%. Infected hosts die after 6 to 10 weeks, releasing millions of long-lasting spores into the environment (*78*).

Temporal monitoring

Our study site was the Aegelsee pond near Frauenfeld, Switzerland, a fishless pond previously described in detail in (*50*), which contains a very large population of *D. magna*. To study the impact of the *P. ramosa* epidemics on the host, we sampled the *D. magna* population throughout its planktonic season (April to early October) for eight consecutive years, monitoring the frequencies of different resistance phenotypes (resistotypes) in the planktonic population. We also used traps to collect *D. magna* resting stages for three seasons

and hatched them under seminatural conditions the following spring. From 2011, a temperature logger was placed in the pond at a water depth of 0.5 m suspended from a buoy near the sampling spot. Water level was recorded at each sampling event.

Field work

Our first sample was collected in early October 2010. From 2011 to 2013, we collected approximately once a month, often a small sample size and without a standardized sampling protocol. From 2014 to 2018, we sampled the *D. magna* population using a standardized protocol every 2 to 4 weeks from early April to early October (more samples during the epidemic). Unless mentioned otherwise, all measurements were done at the deepest location close to the southern bank of the pond.

To monitor prevalence and the evolution of resistance, we sampled planktonic *D. magna* females at each collection date. We scooped the whole depth of the water column with a net (20-cm width and 1-mm mesh opening) to obtain several hundred animals. Samples were kept at 15° to 20°C and transported to the laboratory and processed within 4 hours.

To sample the overwintering resting stages of the population, we collected surface sediment from five locations in the pond once in February 2014, before onset of the natural hatching season. This sample represents the overwintering resting population produced during the active season in 2013. To longitudinally sample the resting stages produced by the *D. magna* population across the season, we used five to nine sediment traps (vertically standing cylinders with 18-cm diameter and 0.4-mm mesh opening) placed on the lake bottom near the deepest part of the lake, and retrieved their content at each collection date during the planktonic season in 2014, 2015, 2017, and 2018. Collected *D. magna* resting stages were hatched in outside containers the following spring after overwintering at 4°C in the dark. Each container contained a hundred ephippia per trap per time point and was monitored for several weeks. We collected hatchlings and cloned them in the laboratory. We measured the resistotype of 20 clonal lines (clones) per trap per time point, resulting in 100 clones per time point.

To obtain an estimate of *Daphnia* density, we used bottles to directly scoop the water from different depths three to five times (from 2011 to 2013). From 2014, we used a plankton net, performing 10 vertical hauls from the bottom of the pond at the deepest point of the lake.

Analysis of field samples

The Aegelsee contains three *Daphnia* species: *D. magna*, *D. pulex*, and *D. curvirostis*. The relative abundance of these species was measured in the laboratory by sorting and counting the density samples using a stereomicroscope. Because *D. pulex* and *D. curvirostis* have similar morphologies, we counted them together and inferred their relative proportions by determining the species in a random subset of 100 animals. We counted the number of males in a subset of 100 *D. magna*.

From each sample, we established clonal (iso-female) lines of about 100 *D. magna* to be used later for resistotype assessment. We estimated the prevalence of infection as described in (*50*), and cured *P. ramosa* infections when they were observed, as otherwise cloning is not possible. *P. ramosa* is the only considerable parasite in this population and was never observed to infect any species other than *D. magna* in this population.

We counted the *D. magna* ephippia retrieved from the traps and overwintered them at 4°C in the dark. In the spring following the collection year (2014, 2015, and 2017), 20 to 100 ephippia (depending on how many were collected at a given sampling time point) from each sampling date were placed in 80-liter containers filled with 30 liters of ADaM (Artificial Daphnia Medium) medium (*79*). Containers were placed outdoors under direct sunlight and checked for hatchlings every second day. We recorded hatching dates and cloned hatchlings in the laboratory. We randomly chose 100 *D. magna* clones equally distributed among replicate traps at each sampling date to assess the resistotypes. To estimate hatching rate, we counted the number of resting stages per ephippium (zero, one, or two) in a subset of 10 to 20 ephippia that were not used for the hatching experiment, in at least two replicates for each collection date.

P. ramosa isolates

We used two *P. ramosa* isolates from our focal population—P20 (*50*) and P21—and three other isolates originating from other populations—C1, C19, and P15 (*55*, *80*). These nonlocal isolates, along with the local isolate P20, were used in previous studies to construct the genetic model of resistance in the host (*37*, *50*, *53*, *54*). In this study, we isolated P21 from our study population by exposing *D. magna* clones to suspended pond sediment. We took one infected female and serially passaged the bacteria from this female three times by infecting females of the same host clone. Spore production in the laboratory followed the protocol described in (*80*).

Resistotype assessment: The attachment test

We determined the resistance phenotype (resistotype) for each *D. magna* clone using the attachment test in (*51*). In short, early in the infection process, bacterial spores will attach to the foregut or the hindgut of susceptible host clones and penetrate the host's body cavity. Spore attachment indicates host susceptibility (S), while absence of attachment indicates host resistance (R). We exposed each individual host to 8000 (C1 and C19) or 10,000 (P15, P20, and P21) fluorescent spores and assessed attachment microscopically. Attachment was judged in each individual as yes or no. We used three replicates of each clone and each parasite isolate, more if the attachment was not clear. This can be the case for P15 and P21, which attach in a different part of the gut as the other isolates (*52*, *55*). Attachment is independent from the environment, making it highly repeatable (*54*, *55*). We obtain a resistotype (R or S) for each host-parasite combination. Across parasite strains, we defined the overall resistotype as the combination of resistance phenotypes to the five individual *P. ramosa* isolates in the following order: C1, C19, P15, P20, and P21 (e.g., a clone susceptible to all isolates will have the SSSSS resistotype). When resistance to a strain is not considered, we use the placeholder ␣, e.g., "RR␣RR resistotype." With time, we were able to include more parasite isolates: From 2010 to 2013, only the resistotypes to C1 and C19 were assessed. In 2014 and 2015, P15 and P20 were added, and all five *P. ramosa* isolates were tested from 2016.

To assess genetic slippage, we calculated the population mean resistance to *P. ramosa* for each sampling time. We assigned a resistance score to each resistotype ranging from zero to one to compare time points when we used different numbers of parasite isolates. For example, a host individual with an RRSRS resistotype was attributed a resistance score of $\frac{3}{5} = 0.6$.

Hatching modeling

To predict resistotype frequencies of sexual offspring of the planktonic *D. magna* population, we used the R package "peas," which generates predictions about the distribution of offspring genotypes

and phenotypes in genetic crosses, based on specified systems of Mendelian inheritance (https://github.com/JanEngelstaedter/peas). We implemented the genetic model of resistance described in (*50*) in the *D. magna*–*P. ramosa* system for our study population. This model includes the genetic architecture of three loci (the B, C, and E loci) that govern host resistance in our study population. The dominant allele at the B locus confers resistance (R) to C19 and susceptibility (S) to C1. The dominant allele at the C locus confers resistance to both the C1 and C19 *P. ramosa* strains, regardless of the genotype at the B locus (epistasis). The E locus contributes to resistance to P20. Resistance is dominant at the C locus (resistance to C1 and C19), whereas resistance is recessive at the E locus (resistance to P20). Homozygosity for the recessive allele at the B and C loci induces susceptibility to P20, regardless of the genotype at the E locus (epistasis). In the present study, we add the genetic architecture of the D locus to the model, which determines resistance to the P15 *P. ramosa* isolate (*55*). Implementation of the model is described in fig. S5 and doc. S1. Implementing this model in the peas package, we calculated the expected resistance genotypes and phenotypes of sexual offspring of each possible mating among parent resistotypes. We assumed different allele frequency scenarios because the known resistotypes of the parents are not sufficient to estimate their exact genotype and allele frequencies, as some alleles can be hidden by dominance and epistasis. We then calculated the expected offspring resistotype frequencies over time corresponding to each of resting stage sample. If the genetic model accurately represents the biology of the system, the expected resistotype frequencies will match those found in the hatchlings from the sediment traps corresponding to the same sampling time. Detailed calculations are described in doc. S2 and fig. S9.

Statistical software

Software used for statistical analyses and graphics are described in doc. S3.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at https://science.org/doi/10.1126/sciadv.abn0051

View/request a protocol for this paper from *Bio-protocol*.

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Acknowledgments: We thank Y. Bourgeois, S. Pichon, S. Gattis, G. Rouseti, J. Hottinger, U. Stiefel, K. Müller, M. Krebs, and D. Vizoso for help in the field and in the laboratory. Members of the Ebert group, J. Stillman, and L. Teixeira provided valuable feedback on the study and the manuscript. We thank two anonymous reviewers whose comments helped to improve and clarify the manuscript. **Funding:** This work was supported by the Swiss National Science Foundation (SNSF) (grant numbers 310030B_166677 and 310030_188887 to D.E.), the Freiwillige Akademische Gesellschaft (FAG) Basel to C.A., and the University of Basel to D.E. and C.A. **Author contributions:** D.E., J.A., and C.A. designed the study. J.A. conducted monitoring

from 2010 to 2013, F.V. conducted monitoring and resting stage hatching in 2014, D.E. conducted monitoring in 2015, and C.A. conducted monitoring and resting stage hatching from 2016 to 2018. D.E. and E.D. monitored resistotype frequency and sexually reproducing animals in 2020 and 2021. J.E. developed the peas R package. C.A. analyzed the data, designed figures, and wrote the manuscript. All authors reviewed the manuscript. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** Data described in this study are available in the Figshare repository at https://figshare.com/articles/dataset/19130495. All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials.

Submitted 1 November 2021
Accepted 4 October 2022
Published 18 November 2022
10.1126/sciadv.abn0051