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Sexual recombination and temporal gene flow maintain host resistance and genetic diversity

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Abstract

Infectious disease can threaten host populations. Hosts can rapidly evolve resistance during epidemics, with this evolution often modulated by fitness trade-offs (e.g., between resistance and fecundity). However, many organisms switch between asexual and sexual reproduction, and this shift in reproductive strategy can also alter how resistance in host populations persists through time. Recombination can shuffle alleles selected for during an asexual phase, uncoupling the combinations of alleles that facilitated resistance to parasites and altering the distribution of resistance phenotypes in populations. Furthermore, in host species that produce diapausing propagules (e.g., seeds, spores, or resting eggs) after sex, accumulation of propagules into and gene flow out of a germ bank introduce allele combinations from past populations. Thus, recombination and gene flow might shift populations away from the trait distribution reached after selection by parasites. To understand how recombination and gene flow alter host population resistance, we tracked the genotypic diversity and resistance distributions of two wild populations of cyclical parthenogens. In one population, resistance and genetic diversity increased after recombination whereas, in the other, recombination did not shift already high resistance and genetic diversity. In both lakes, resistance remained high after temporal gene flow. This observation surprised us: due to costs to resistance imposed by a fecundity-resistance trade-off, we expected that high population resistance would be a transient state that would be eroded through time by recombination and gene flow. Instead, low resistance was the transient state, while recombination and gene flow re-established or maintained high resistance to this virulent parasite. We propose this outcome may have been driven by the joint influence of fitness trade-offs, genetic slippage after recombination, and temporal gene flow via the egg bank.

Keywords *Daphnia dentifera* · *Metschnikowia bicuspidata* · Heritability · Cyclical parthenogenesis · Storage effect · Gene flow

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Introduction

Epidemics threaten many host populations–for organisms as different as blue whales and bacteria, virulent infectious disease can drive population decline and, in some cases, extirpation or extinction (Smith et al. 2006; Warner 1968; Wyatt et al. 2008). Fortunately, most species can evolve resistance if their populations contain enough standing genetic variation (Betts et al. 2016; Bonneaud et al. 2011; Duffy & Hall 2008; Duffy & Sivars-Becker 2007; Laine 2006). Resistance can evolve on ecological timescales, potentially protecting host populations from some of the harms of virulent parasites (Betts et al. 2016; Duncan & Little 2007; Edeline et al. 2008; Lohse et al. 2006; Penczykowski et al. 2011; Strauss et al. 2017).

Rapid evolution of host resistance has drawn scientific interest in the past, but most perspectives focus on resistance evolution within a growing season (Betts et al. 2016; Duncan & Little 2007; Edeline et al. 2008; Lohse et al. 2006; Penczykowski et al. 2011; Strauss et al. 2017) or across a span of multiple years to decades (Bonneaud et al. 2011; Dybdahl & Lively 1998; Gandon et al. 2008). Relatively little attention has been paid to how population resistance translates across an annual cycle of dormancy and growth (though see Frickel et al. 2018). Yet, this scenario applies to organisms across the tree of life that engage in cyclical extinction-repopulation dynamics. Adaptation in these populations is modulated not only by evolution within a season, but also by annual genetic and phenotypic changes that occur during the (often sexual) production of dormant stages, and during temporal gene flow (i.e., recolonization) from the germ bank (Decaestecker et al. 2009; Gyllström & Hansson 2004). We need to broaden our understanding of how germ banks alter rapid evolutionary dynamics if we want to predict if resistance evolution will provide lasting protection in seasonal host-parasite systems.

Recombination and gene flow are two mechanisms that can maintain the genetic variation necessary for natural selection, but they can also act in opposition to rapid local adaptation (Forde et al. 2007; García-Ramos & Kirkpatrick 1997; Hendry et al. 2001; Lenormand 2002). Recombination can lead to genetic slippage, wherein the population mean for a phenotype under selection will shift away from a new population optimum, and instead restore a previous phenotype distribution with lower mean fitness (Lynch & Deng 1994). Furthermore, cyclical sexual reproduction (i.e., cyclical parthenogenesis) is frequently associated with the formation of diapausing propagules (Decaestecker et al. 2009; Gyllström & Hansson 2004). These propagules can persist through harsh environmental conditions and, due to variable hatching rates, collect over time to create a genetic archive (or germ bank) of past populations (e.g., Cáceres 1997; Cohen 1966; Jones & Lennon 2010; Locey et al. 2016; Warr et al. 1993). Gene flow out of the germ bank reintroduces allele combinations from past populations; this reintroduction can oppose recent adaptation. Therefore, a population that rapidly evolves resistance to a virulent parasite during an asexual phase may then experience a "reversal" to a more susceptible state due to sexual recombination and/or temporal gene flow from the germ bank.

Do these mechanisms – recombination and temporal gene flow – impact resistance in wild host populations? To explore this question, we sampled two populations of germ banking cyclical parthenogens at the end of one active season and the beginning of the next. We disentangled the effects of recombination and gene flow on host resistance in two phases. First, we compared the resistance phenotypes and the genotypes of asexually produced animals to their sexually produced offspring. Then, we compared resistance phenotypes and genotypes of animals hatched from sexually produced diapausing propagules

(which entered the egg bank in the fall) to animals from the following spring (likely repopulated from the egg bank). Due to fitness (fecundity) costs associated with resistance in this system, we expected host populations to be susceptible to the pathogen unless a recent epidemic had selected for increased resistance. Then, if a population had high mean resistance in the fall, we expected this resistance to decrease after sexual recombination due to genetic slippage toward the pre-epidemic (lower) mean resistance. As for the egg bank effect, if there was no temporal gene flow (i.e., no gene flow from resting eggs created in previous years), then all springtime animals will have hatched from resting eggs produced the previous fall. Therefore, the null expectation would be the resistance of the egg bank population would match that of the sexually produced offspring collected the previous fall. We expected our populations to deviate from this null hypothesis and show evidence of temporal gene flow. However, we acknowledge that these predictions are based on scenarios where only the listed factors operate; natural ecosystems are far more complex. A variety of factors, most notably predation regimes, have the potential to lead to more complicated dynamics, as we discuss below. Overall, this study helps us understand how two common phenomena — sexual recombination and gene flow from the egg bank – combine to impact host resistance across years in cyclically parthenogenetic germ banking species.

Study system

The host, a facultatively sexual parthenogen: Daphnia is a genus of freshwater planktonic crustacean that hosts multiple parasites. Its short generation times and ability to reproduce asexually make it a particularly tractable study system (Ebert 2005; Stollewerk 2010), and allow for rapid evolution through clone competition. Our study focused on *Daphnia dentifera,* a daphniid commonly found in freshwater lakes across the midwestern United States (Tessier & Woodruff 2002). *D. dentifera* is cyclically parthenogenetic; animals hatch from the resting egg bank in spring then reproduce asexually (clonally). During fall, female *D. dentifera* produce male offspring and switch to sexual production of resting eggs (Gowler et al. 2021). After the release of resting eggs, the active population dies off for winter.

The parasite and fitness components: This host species can suffer epidemics of *Metschnikowia bicuspidata* (hereafter: *Metschnikowia*), a virulent fungal pathogen of *D. dentifera* that reduces host lifespan up to 50% (Clay et al. 2019) and reduces host fecundity by approximately 25% (Auld et al. 2012; Duffy & Hall 2008). Fish also selectively prey on *D. dentifera* infected by *Metschnikowia*, further increasing their mortality rate (Duffy & Hall 2008). Infection occurs when the host consumes fungal spores while filter-feeding in the water column. The spores pierce the host's gut wall and enter the hemolymph, where they enter a rapid growth phase and fill the host's body cavity with transmission stages (Metschnikoff 1884; Stewart Merrill & Cáceres 2018). Upon host death, spores enter the water column (Ebert et al. 2000).

Resistance and trade-offs: Resistance to *Metschnikowia* is highly variable in *D. dentifera* (Auld et al. 2013; Duffy & Sivars-Becker 2007). Body size, feeding rate, and gut thickness in *D. dentifera* correlate with resistance to *Metschnikowia* (Hall et al. 2010; Stewart Merrill et al. 2021). Additionally, body size, feeding rate, and fecundity are positively correlated in *D. dentifera* (Burns 1969; Hall et al. 2010, 2012). Hence, fecundity in *D. dentifera* trades off against resistance to *Metschnikowia*. This fitness trade-off may help explain complex evolutionary outcomes in this host-parasite system, such as disruptive selection for either very high or very low resistance (Duffy et al. 2008). It also raises the possibility that predation regimes might influence resistance, as visual predators such as fish select for

smaller body sizes (Galbraith 1967; Kitchell & Kitchell 1980; Wells 1970) while gape-limited *Chaoborus* larvae select for larger body sizes (Pastorok 1981; Spitze 1991). Finally, we know fungal epidemics can spur a rapid shift in host resistance within a single active (primarily asexual) season for *D. dentifera* (Duffy et al. 2008, 2009, 2012; Duffy & Hall 2008; Duffy & Sivars-Becker 2007). However, little is known about how such evolutionary shifts in host resistance translate from one active season to the next.

Methods

Field sampling

We collected *Daphnia* from two lakes, Midland and Hackberry Lakes, late in the year (December 2015) and in the following spring (April/May 2016). Midland and Hackberry Lakes are both dimictic lakes located in Greene County, Indiana, USA. These lakes were used because *Metschnikowia bicuspidata* is a common parasite of *D. dentifera* in Greene County, Indiana, with prevalence as high as 60% (Shaw et al. 2020). In 2015, Hackberry Lake had very few late-stage infections (annual maximum prevalence = 0.05%), while Midland Lake had a moderate epidemic (annual maximum prevalence = 17%; S.R. Hall, *unpublished data*).

In December 2015, we collected uninfected female *D. dentifera* bearing ephippia (i.e., sexually produced resting eggs) from the two study lakes. Each subsample was taken from a single whole-water column vertical net tow. Since parasitized animals do not spatially segregate (Hall et al. 2005), this tow should provide a representative sample of the resistance trait among sexually reproducing individuals. However, if clones vary in their propensity to reproduce sexually (as in *D. pulicaria;* Cáceres & Tessier 2004a, Cáceres & Tessier 2004b), our sample of sexually producing females might have captured genotypes that invest more in sexual reproduction. Ephippial females ('parents') released their ephippia in the laboratory. We hatched the ephippia, then maintained cultures of those offspring in standardized conditions (6 individuals/30 mL water, 20 °C, 16:8 light/dark cycle, fed 10^6 cells/mL *Ankistrodesmus falcatus* 4 times weekly). Those conditions maintained isofemale (clonal) lines of each parent and their offspring asexually. The following spring (i.e., in April and May 2016), we sampled active populations (assumed to be newly hatched from the egg bank) with a single whole-water column tow. We then maintained clonal lines of these animals using the same methods. This yielded three sets of clones per lake—'parents', 'offspring', and 'egg bank'—all maintained under standardized conditions for resistance (phenotypic) assays and genotyping.

Resistance assays

Infection assays followed standard protocols (Duffy & Sivars-Becker 2007), with the experiment split into 4 blocks due to logistical constraints. Animals were maintained in standardized conditions to standardize maternal effects. Additionally, the third (or later) clutches were used to propagate the next generation. We maintained this procedure for at least three generations until we accumulated 40 or more individuals of the same age for each clonal line.

For each clonal line, five *Daphnia* (6–8 days old) were distributed to each of eight 150 mL beakers (= 40 animals per clone) with 100 mL of filtered lake water. Each

beaker received 1 mL of 1×10^5 cells/mL of *Ankistrodesmus falcatus* phytoplankton as a food source. This ration encouraged spore uptake during exposure to 250 cells/mL of *Metschnikowia* for 24 h. The *Metschnikowia* spores were from the "Standard" isolate from Baker Lake (Barry County, MI, USA). Resistance to *Metschnikowia* is a quantitative trait, and there is no evidence for host-parasite genotype specificity (Duffy & Sivars-Becker 2007), so observations from this one fungal strain should generalize to the *Metschnikowia* species. Exposures were ended by transferring animals to new beakers with 100 mL of spore-free filtered lake water. Animals were fed 2 mL of 1×10^5 cells/mL of *Ankistrodesmus falcatus* 4 times per week and kept at 20 °C and on a 16:8 light/dark cycle throughout the experiment with weekly transfer to fresh water. We examined animals for visible terminal infection 11–12 days after exposure, a time point when infected hosts show symptoms but have not yet died from infection (Auld et al. 2012).

Genotyping

We also characterized the multilocus genotype of each clone to track shifts in genotypic diversity. We genotyped clones used in the assays for three reasons. First, since the host reproduces asexually through much of the year (Gowler et al. 2021), multiple isolated individuals might have belonged to the same genotype. Second, populations might shift genetically between the three periods without changes in the resistance phenotype. Third, genetic identities were needed to evaluate recombination, gene flow, and population genetic structure. Hence, we genotyped each clonal line collected.

We used six microsatellites from eight previously published sets of primers according to their map position, ease of scoring and allelic diversity (primers: Dgm105, Dgm106, Dgm107, Dgm109, Dgm112, Dgm113; Table S1; based on Fox 2004). However, Dgm 107 did not provide us with any detectable peaks, leaving us with five microsatellite loci. Each locus was assigned one of four different fluorescent labels (6FAM; MAX; ATTO; ROX, Integrated DNA Technologies) in such a manner that no two markers with the same fluorescent dye had overlapping allele size ranges. We extracted DNA from a single uninfected animal from each clonal line using the standard protocol included in the DNeasy Blood & Tissue Kit (Qiagen).

Polymerase chain reaction (PCR) amplifications were performed in 96 well plates (one reaction per well) using QIAGEN® Multiplex PCR kit. PCR reactions were carried out in a final volume of 50 µL with 25 µL of 2 × Qiagen multiplex mastermix (QIAGEN, Hilden, Germany), 0.2 µM of each forward and reverse primer pair (for a final volume of 1.2 µM), and < 1 µg of DNA, with the remaining difference in volume made up by RNase-free water. Amplification conditions were: 95 °C (15 min), then 35 cycles of 94 °C (30 s) / 58 °C (3 min) / 72 °C (1:30 min), and a final extension at 72 °C for 10 min. For genotyping, 1 µl of diluted (1:200) PCR products were added into capillary electrophoresis loading plates containing 11 µl Hi-Di formamide and a LIZ500 size standard. Fragment analysis was performed by the University of Michigan DNA sequencing core, and fragment lengths were read using GeneMapper (ThermoFisher Scientific).

We wanted to see if shifts in population genetics reflected the shifts in population resistance and also wished to match genotype and phenotype data for each clone. To quantify the impact of recombination on genetic variation, we compared the genotypic evenness and diversity of parents with their sexually recombinant offspring. Similarly, we quantified how temporal gene flow impacted genetic variation in a lake by comparing genotypic evenness and diversity of that lake's sexually recombinant offspring to the corresponding spring egg

bank population. We used the *poppr* package (version 2.0.2) in R (version 4.0.0) to measure genotypic richness (number of multilocus genotypes (MLGs)) and genotypic diversity using three indices: Shannon–Wiener index (H), Stoddart and Taylors (G), and Simpson (λ). Using all three indices allowed us to look for shifts in population diversity across multiple metrics (Fig. 1). We also quantified MLG evenness to help detect dominant genotypes and used clone-corrected data to calculate the index of association (I_A, a measure of linkage disequilibrium; Table S2) for each group.

Due to logistical constraints, we could only assay a fraction of each population for the resistance trait. Moreover, due to a lab accident, we lost six isofemale lines that had been used in the resistance assays before genotyping. Therefore, we could not assign a resistance phenotype to every genotype.

Statistical analysis

For the main statistical analysis, resistance was calculated as the proportion of uninfected animals for each *Daphnia* clonal line. To measure the effect of sexual recombination on the resistance phenotype, we compared resistance to *Metschnikowia* between parents and their sexually produced offspring by modeling the number of uninfected hosts per beaker using a binomial GLMM fit by maximum likelihood (LaPlace approximation) with parent vs.

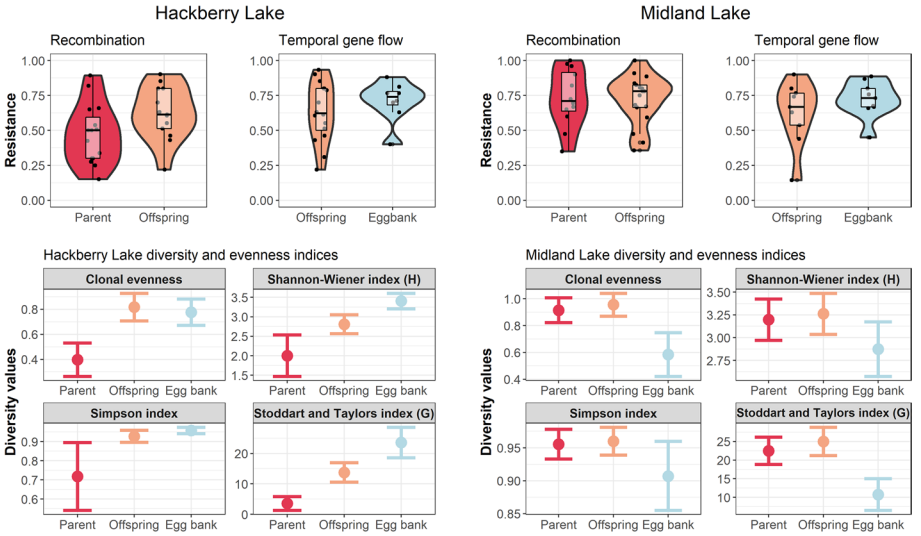

Fig. 1 Resistance and genotypic diversity significantly increased after recombination occurred in Hackberry Lake, but remained constant in Midland Lake. Figure 1A and C show mean resistance of isofemale lines collected at two time points: ephippial females in December 2015 ('Parent') and offspring hatched from those ephippia ('Offspring'). Figure 1B and D show mean resistance of offspring hatched from ephippia produced in December 2015 ('Offspring') and individuals collected in Spring 2016 after the active population was refounded from the egg bank ('Egg bank'). Phenotype comparisons are paired by experimental blocks (one block shown in A, another in B, etc.), so resistance in parents and egg bank animals cannot be directly compared. The violin plot outlines illustrate kernel probability density, i.e., the width of the shaded area represents the proportion of the data located there. Fig E and F show the observed diversity measures and bootstrapped 95% confidence intervals. The bootstrapped estimates often skew from the observed measures, and confidence intervals were centered around the observed diversity measures as recommended by Grünwald et al. 2017

offspring as a fixed effect using the lme4 package (v1.1–26; (Bates et al. 2014)). To incorporate the dependency among observations from the same clonal line and between parent–offspring pairs, we included 'clone' (i.e., whether individuals were the same multilocus genotype) and 'family' (i.e., whether the parent had produced the particular offspring) as random effects.

To measure the effect of temporal gene flow on the resistance phenotype, we analyzed the resistance of sexually produced offspring vs. egg bank individuals. If there was no temporal gene flow from resting eggs, then we expected that all springtime animals had hatched from resting eggs produced during the previous fall. Therefore, our null expectation was that the resistance of the egg bank population would match that of the sexually produced offspring collected the previous fall; we tested this statistically with a binomial GLMM with number of uninfected hosts per beaker as the response variable with time point ('offspring' vs. 'eggbank') as a fixed effect and 'clone' as a random effect.

Due to logistical constraints, we could not run all resistance assays simultaneously. Hence, we prioritized grouping based on the comparisons of interest. For example, since we were not interested in comparing between lakes, we ran clones from the different lakes in different blocks. Consequently, we only directly compared groups exposed within the same block. The one exception is for Hackberry, where we compared resistance in Hackberry offspring to Hackberry egg bank animals using data from two exposure blocks after confirming no block effects existed. Finally, we calculated narrow-sense heritability for each lake population. To calculate it, we regressed mean offspring resistance vs. mean parent resistance, where h^2 is twice the slope of the regression (Falconer, 1981). If a parent had produced two offspring we used the mean resistance scores of both offspring. Because this analysis required that we have estimates of heritability for both the parent and at least one offspring, there are fewer parent (and offspring) individuals included in this analysis than in the other analyses.

Results

Impact of sexual reproduction on mean resistance and genetic diversity

Mean resistance of the population increased after sexual recombination in Hackberry Lake (from 0.47 in parents to 0.62 in offspring; $z = 2.0$, $p = 0.046$; Fig. 1a). Genotypic diversity also increased; the moderate genotypic diversity of parents (Simpson index = 0.72, 95% CI: 0.54 – 0.90; Fig. 1e) increased significantly in their sexually produced offspring (Simpson index = 0.93, 95% CI: 0.89 – 0.96; Fig. 1e; changes were qualitatively the same for the Shannon–Wiener and Stoddart & Taylors indices; Table S2). Notably, one relatively susceptible and dominant (53%) genotype drove both lower population-level resistance and genotypic diversity in Hackberry parents (MLG.50; mean resistance = 0.36; Fig. 2 and S1). This same genotype was also found in sexually produced offspring, though at a lower frequency (14%). In most cases (19 out of 26), offspring had a different multilocus genotype than their parent; in seven cases, a parent and its offspring shared identical MLGs. Narrow-sense heritability (h^2) of resistance was 0.52 in the Hackberry population (Fig. 3).

In contrast, sexual recombination had no effect on the mean resistance in Midland Lake. This population had relatively high resistance in the fall 'parent' population (0.74) and this did not change for 'offspring' (i.e., after sexual recombination; 0.72; $z = 0.26$, $p = 0.79$; Fig. 1c). Similarly, genotypic diversity of this population was already high in parents and

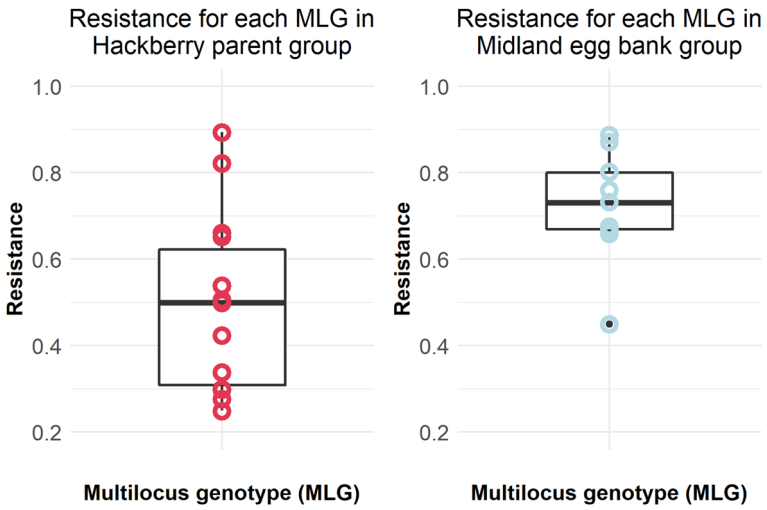

Fig. 2 Multilocus genotype 50 (i.e., MLG.50, left plot) was the most prevalent genotype in the Hackberry parent lake-group. It was also on average less resistant to infection by *Metschnikowia bicuspidata* compared to the majority of other, co-existing genotypes. Multilocus genotype 57 (i.e., MLG.57, right plot) was the most prevalent genotype in the Midland egg bank lake-group even though it was not detected in the fall prior. This is evidence of temporal gene flow, i.e., that resting eggs produced during earlier years help recolonize lakes in the spring. MLG.57 does not have an extremely susceptible or resistant phenotype compared to other, coexisting genotypes

did not significantly change after sexual recombination (Simpson index: parents = 0.96, 95% CI: 0.93 – 0.98; offspring = 0.96, 95% CI: 0.94 – 0.98; Fig. 1f; again, all three diversity metrics had qualitatively consistent results). Furthermore, in Midland Lake, no genotype obviously dominated after sexual recombination, as 24 out of 30 offspring had unique MLGs (Fig. S1). Narrow-sense heritability (h^2) of resistance was 0.33; this lower heritability in Midland relative to Hackberry was likely due to less variation in resistance in Midland (Fig. 3).

Mean resistance and genetic diversity after hatching from the egg bank

The Hackberry Lake population maintained high resistance after gene flow from the egg bank (Fig. 1b): mean resistance in fall offspring (0.63) did not differ from that of spring egg bank clones (0.71; $z = -0.02$, $p = 0.98$). However, genotypic diversity increased between fall offspring and the spring egg bank clones (Fig. 1e). While this increase was not statistically significant for the Simpson index (fall offspring = 0.93, 95% CI: 0.89 – 0.96; spring egg bank = 0.96, 95% CI: 0.94 – 0.98), this index was near its upper bound, and the increase was significant for the other two diversity indices (Shannon–Wiener: fall offspring = 2.81, 95% CI: 2.56 – 3.06; spring egg bank = 3.41, 95% CI: 3.21 – 3.60; Stoddart and Taylors: fall offspring = 13.76, 95% CI: 10.48 – 17.05; spring egg bank = 23.56, 95% CI: 18.41 – 28.78). The Hackberry parent population also stood out for having unusually high linkage disequilibrium, as quantified by the index of association ($I_A = 0.54$ for Hackberry parents *vs.* < 0.16 for all other lake-time point combinations; Table S2); however, we

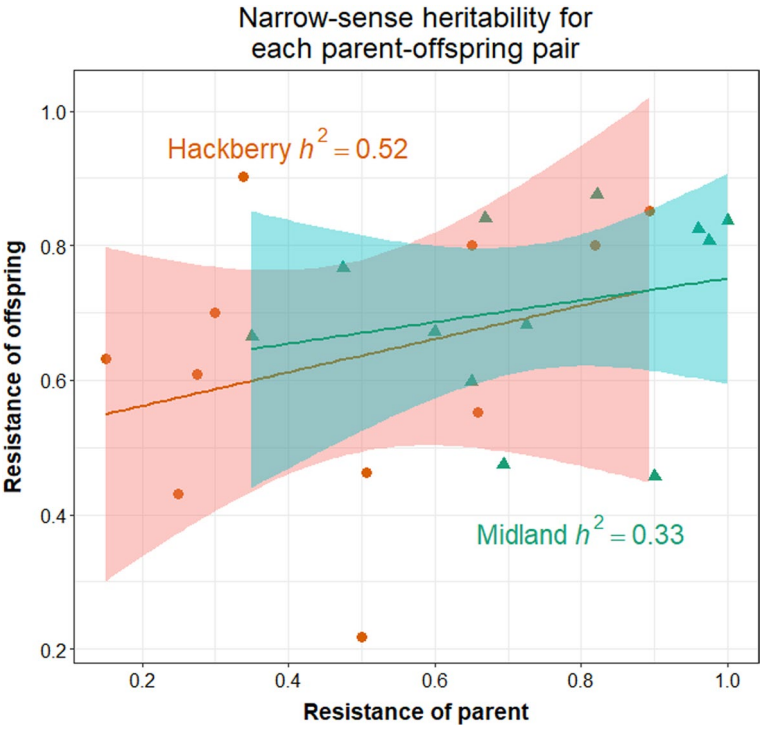

Fig. 3 Both populations showed moderate heritability of resistance (h^2); the Hackberry Lake population (orange) had an h^2 of approximately 0.52, while the Midland Lake population (green) had h^2 of approximately 0.33. Midland scored lower due to low variance (i.e., high similarity) in resistance for both parents and offspring of the population. Narrow-sense heritability for both lake populations was found by doubling the slope of the linear regression of parent vs. offspring resistance

note that the relatively small number of loci in our study means I_A should be interpreted with caution.

In Midland Lake, the population also maintained high resistance after gene flow from the egg bank (Fig. 1d), similar to Hackberry Lake. However, in contrast, genotypic diversity in Midland Lake did not increase for egg bank clones (Fig. 1f); instead, the genotypic diversity of the egg bank clones was the same as (Simpson: fall offspring = 0.96, 95% CI: 0.94 – 0.98; spring egg bank = 0.91, 95% CI: 0.85 – 0.96; Shannon–Wiener: fall offspring = 3.26, 95% CI: 3.05 – 3.48; spring egg bank = 2.87, 95% CI: 2.56 – 3.19) or lower than (Stoddart and Taylors: fall offspring = 25.00, 95% CI: 21.37 – 28.63; spring egg bank = 10.77, 95% CI: 6.25 – 15.28) that of the fall offspring.

Discussion

How do recombination and gene flow impact population resistance across a seasonal cycle of extinction and recolonization? In our system, resistance and fecundity trade off as a result of their joint relationships with host feeding rate (Hall et al. 2010; though this does not happen in all populations: Auld et al. 2013). Given this trade-off, we expected

populations would evolve toward higher susceptibility (due to its fecundity advantages) unless an epidemic had recently selected for resistance. If an epidemic did occur, we expected resistance to increase temporarily. Then, due to sexual recombination and temporal gene flow, we expected the population to shift back towards the recent susceptible state. Contrary to our expectations, susceptibility was the transient state, with recombination and gene flow restoring and/or maintaining high resistance. Moreover, we expected that fall offspring would show greater genotypic diversity than their parents due to the effects of sexual recombination; this was observed in Hackberry Lake but not in Midland, where genotypic diversity of parents was already very high. We further expected that the eggbank clones would have higher diversity than the fall offspring, since we anticipated hatching of individuals produced across multiple years; again, this was observed in Hackberry but not in Midland. Given that logistical constraints rendered it impossible to quantify selection (due to parasitism and/or other selective forces) throughout the season, we cannot tell whether the differences in Hackberry vs. Midland are driven by the difference in infection prevalence in those two lakes. However, our cross-season study confirms that sexual recombination and temporal gene flow are both important players in determining inter-annual variation in host resistance in this study system, and that this area of inquiry warrants further study.

Our data might indicate stronger selection in Hackberry Lake (which did not have an epidemic) than in Midland. *D. dentifera* collected during the fall in Hackberry had the lowest resistance, lowest genotypic diversity, and the highest index of association of either population at any sampling time point in the study. Together, these results suggest recent selection favoring increased susceptibility in this population. In contrast, Midland Lake, which had an epidemic with an annual maximum prevalence of 17%, had high genetic diversity and low linkage disequilibrium (Table S2). Low linkage disequilibrium suggests random genetic shuffling, while high linkage disequilibrium can be a sign of strong selection, very high clonal reproduction, and/or genetic drift (Slatkin 2008). Genetic drift seems unlikely in these very large populations, leaving high clonal reproduction and strong selection as possible explanations. Both are possible.

Both Midland and Hackberry had high clonal reproduction during summer and into fall (S.R. Hall, unpubl. data). When *Daphnia* shift from asexual to sexual reproduction, males first appear in the population, followed by ephippial (sexual) females. In 2015, males were not observed in these populations until October, and sexual females were not observed until the end of October in Hackberry and beginning of November in Midland (S.R. Hall, unpubl. data). Midland Lake invested more heavily in sexual reproduction, with 40% of the population being males or ephippial females in November *vs*. 21% in Hackberry. Together, this suggests that both populations had high levels of clonal reproduction, but that the impact of this in Midland may have been somewhat mitigated by a greater shift to sexual reproduction. However, given that the first ephippial females appeared in the population right around when we collected ephippial females for this study, it is highly unlikely that any of the 'parents' that we collected for this study were the result of sexual recombination during 2015, as there would not have been enough time for those ephippia to be produced, released, hatch, and for the individual to reach adulthood prior to us collecting our samples. Thus, it is likely that the strength of selection differed between these two lakes.

Why would selection be stronger in the lake that did *not* experience an epidemic of a highly virulent parasite? While prior work has focused particularly on a resistance-fecundity trade-off, myriad other factors could influence the selective environment. Indeed, the genotype data suggests this was the case. In Hackberry, one highly susceptible genotype, MLG.50, dominated in the fall population while other susceptible genotypes that

were present remained rare. Perhaps, then, not all susceptible genotypes enjoyed fitness advantages in this population. Overall, multiple relationships link traits such as resistance, fecundity, predation, and resource acquisition with body size, with selective pressures shifting throughout the active season. For example, if faster-feeding genotypes are more susceptible, then they are at a disadvantage when food quality is low. This scenario could arise because *D. dentifera* experience trade-offs in their ability to exploit high- versus low-quality food (Hall et al. 2012), and food quality changes throughout the summer and fall (Hall et al. 2009). Another possible mechanism is predation by invertebrate and vertebrate predators, which also correlates with body size of *D. dentifera* (Strauss et al. 2016) and can therefore indirectly select on host resistance. These mechanisms are at play in other host-parasite systems, as well. Resistance generally comes with a cost to fitness (Roy & Kirchner 2000; Simms & Triplett 1994) and trade-offs between resistance and fecundity, longevity, and rate of maturation are found across a diversity of hosts (Buckling & Brockhurst 2012; Gwynn et al. 2005; Kraaijeveld et al. 2002; Langand et al. 1998). Additionally, ecological interactions such as predation and mate selection mediate the strength of these trade-offs in these other systems (Clayton et al. 2015; Møller 2008; Toor & Best 2015), similar to *Daphnia*. Ultimately, understanding the drivers of resistance evolution in any host-parasite system will require understanding the impacts of multiple selective agents and ecological processes.

Alternatively, it's possible that the pattern actually reflects parasite mediated selection in the lake that experienced an epidemic, Midland. Prior to the parasite outbreak, the selection pressures in Midland may have been similar to those in Hackberry, which would have favored a relatively susceptible genotype such as MLG.50. Once the epidemic began, we would expect selection against susceptible genotypes, which would lead to a 'parent' population with high mean resistance but that still had relatively high diversity (that is, a scenario that looks like the Hackberry parents in Figure S1, except missing the one highly dominant, susceptible genotype). Distinguishing between these two scenarios (stronger selection in Hackberry *vs*. Midland) will require future studies that monitor changes in genetic composition more frequently while also tracking ecological dynamics.

If mating was random, why did sexual recombination increase resistance in Hackberry? One possible explanation involves genetic slippage. Depending on the mode of gene action and the selection function, the action of segregation and recombination can cause the mean phenotypic value of a population to move in a direction contrary to selection (Ameline et al. 2021; Lynch & Deng 1994). This has been seen in *Daphnia pulicaria* as well as facultatively sexual rotifers (Becks & Agrawal 2012), *Chlamydomonas* (Kaltz & Bell 2002), and yeast (Goddard et al. 2005). Non-random mating may also play a role, as noted in past work (Duffy et al. 2008), though no studies have directly detected assortative or otherwise non-random mating in *Daphnia*. Chemical signals or differences in habitat use could increase the likelihood of non-random mating, which could shift the trait distribution of the population (in a direction dependent on whether similar or dissimilar animals mate more frequently). Such non-random mating has been described in a wide range of taxa and has significant ecological and evolutionary consequences (Crespi 1989; Janicke et al. 2019; Jiang et al. 2013).

Temporal gene flow out of the diapausing egg bank increased genetic diversity in Hackberry but, if anything, decreased it in Midland. In both cases, we hypothesize that this was due to the hatching of genotypes that had been produced in previous years. Two lines of evidence support this claim. First, in Hackberry Lake, the susceptible genotype MLG.50 hatched out of the egg bank at a different frequency than it was deposited into it (Fig S1), resulting in a more diverse (if equally resistant) population. We hypothesize that the novel resistant clones that

hatched from the egg bank may have been produced during previous years with large epidemics; unfortunately, no long-term monitoring data exists for Hackberry Lake so we cannot test this hypothesis with existing data. Second, during the 'egg bank' phase a new clone became common in Midland (MLG.57, which made up 12 out of 48 (25%) of Midland egg bank clones; Figs. 2 and S1). This clone was not present in either the 'parent' or 'offspring' samples, suggesting it emerged from the egg bank after having been deposited in years past. This clone had moderate resistance. An additional possibility for the Midland Lake result, however, is that the dominance of MLG.57 in the spring 'egg bank' clones may have resulted not from it being dominant in the egg bank but, rather, from it *not* investing in sexual reproduction. In other species of *Daphnia*, some genotypes invest less in sexual reproduction (Spaak 1995; Zeis et al. 2010; Tessier and Caceres 2004), instead maintaining populations in the water column even through unfavorable conditions – this strategy is similar to a plant that invests in vegetative growth rather than producing seeds. While we have not found *D. dentifera* under the ice, our winter sampling has been quite limited. It would be interesting to better assess whether some *D. dentifera* individuals persist in the water column through winter and, if so, if genotypes vary in their propensity to do so.

In conclusion, our findings highlight the importance of recombination and germ banks in maintaining genetic diversity in asexual or cyclically parthenogenetic organisms. Furthermore, we found that both factors likely underpin interannual dynamics of resistance in germ banking organisms. Future studies monitoring the genotypic and phenotypic values of populations across multiple sequential (seasonal) extinction-recolonization events, while also tracking epidemiological dynamics, would help determine the generality of our findings while better connecting rapid interannual selection dynamics with longer-term evolution.

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Author's contributions MAD and SRH conceived of the study. CDG did field collections with assistance from SRH. CDG hatched resting eggs. KDM and HZ completed phenotypic assays. HZ conducted molecular work with guidance from KDM. KDM and MKD analyzed molecular data. KDM wrote the manuscript with assistance from HZ and in consultation with MAD; all authors edited the manuscript.

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Declarations

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