Simulated climate change, epidemic size and host evolution across host-parasite populations

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Abstract

Climate change is causing warmer and more variable temperatures as well as physical flux in natural populations, which will affect the ecology and evolution of infectious disease epidemics. Using replicate semi-natural populations of a coevolving freshwater invertebrate-parasite system (host: *Daphnia magna*, parasite: *Pasteuria ramosa*), we quantified the effects of ambient temperature and population mixing (physical flux within populations) on epidemic size and population health. Each population was seeded with an identical suite of host genotypes and dose of parasite transmission spores. Biologically reasonable increases in environmental temperature caused larger epidemics, and population mixing reduced overall epidemic size. Mixing also had a detrimental effect on host populations independent of disease. Epidemics drove parasite-mediated selection, leading to a loss of host genetic diversity, and mixed populations experienced greater evolution due to genetic drift over the season. These findings further our understanding of how diversity loss will reduce the host populations' capacity to respond to changes in selection, therefore stymying adaptation to further environmental change.

INTRODUCTION

The earth's climate is changing, giving rise to warmer temperatures and more variable weather (Coumou & Rahmstorf, 2012). Heat waves, droughts and floods are more common and are driving shifts in the severity and distribution of infectious disease. Warming can increase parasite development rate and transmission stage production (Poulin, 2006), as well as overall transmission rate (Kilpatrick *et al.*, 2008), whereas increased variance in temperature can independently drive shifts in parasite growth and transmission (Murdock *et al.*, 2016). Temperature changes can also differentially affect the phenology of hosts and parasites in such a way to either increase or reduce transmission. For example, warming increases the likelihood and severity of trematode infections in snails, but reduces the likelihood of onward trematode transmission (and thus epidemic size) to the definitive amphibian host (Paull & Johnson, 2014). Physical flux resulting from droughts, floods *etc.* could also have profound effects on disease by increasing contact rates between hosts and parasites and thus parasite transmission rate (May & Anderson, 1979). It is clear that the effects of climate change on infectious diseases are often complex, and can shape disease dynamics in sometimes unpredictable and counter-intuitive ways (Parmesan & Yohe, 2003; Lafferty, 2009).

By affecting epidemic size, climate change could have profound effects on host populations. Epidemics can reduce population densities in susceptible hosts, and thus drive parasite-mediated selection (Auld *et al.*, 2013) and population genetic change (Duncan & Little, 2007; Thrall *et al.*, 2012). For example, larger epidemics can exert stronger directional selection for increased host resistance, stripping genetic variation from populations (Obbard *et al.*, 2011). Patterns of epidemic size, parasite-

mediated selection and host genetic diversity are thus intrinsically linked. This is important, because genetic diversity determines how a host population can respond to subsequent disease epidemics (Altermatt & Ebert, 2008; King & Lively, 2012), as well as other selective pressures. Indeed, genetic diversity is the fuel for adaptation, so low diversity populations are vulnerable to extinction when there is a change in selection pressures (Lande & Shannon, 1996). By influencing epidemic size, environmental variables such as ambient temperature and physical flux are pivotal in shaping eco-evolutionary feedbacks and long-term health in natural populations (Vander Wal *et al.*, 2014).

The effects of biotic and abiotic environmental conditions on individual disease phenotypes have been effectively dissected using controlled laboratory experiments in numerous systems (McNew, 1960; Salvaudon *et al.*, 2009; Wolinska & King, 2009; Vale, 2011). However, in order to identify the mechanisms through which climate change shapes the evolution of disease more generally, we must incorporate ecological complexity to determine how these individual phenotypes scale up to the population level. Population-level studies are commonly observational, so the benefit of having a realistic assessment of disease patterns in ecologically complex conditions is often accompanied with the cost of not being able to uncover the mechanisms that drive those patterns. The challenge is to incorporate realistic ecological complexity whilst retaining a degree of experimental control. Semi-natural experimental populations - mesocosms - provide an excellent opportunity to do this (Benton *et al.*, 2007) because they allow natural variation in season, and thus photoperiod and temperature, yet are easily subject to experimental manipulation.

Here, we present the results of an outdoor mesocosm experiment designed to test the following hypotheses: that the mean and variance in temperature as well as

physical flux (population mixing) affects: (1) the timing and severity of disease epidemics; (2) the strength and consistency of parasite-mediated selection; and (3) the genetic diversity of host populations. We established twenty replicate outdoor mesocosms of the freshwater crustacean, *Daphnia magna* and its sterilizing bacterial parasite, *Pasteuria ramosa*. *Daphnia* have a remarkable reproductive biology that means they can reproduce both sexually and asexually. By propagating *Daphnia* genotypes asexually, we were able to seed each mesocosm with an identical suite of *Daphnia* genotypes as well as spores from the same starting parasite population. Whilst the genetic composition of hosts and parasites was the same across mesocosms, the ambient temperature and level of population mixing varied. This experimental system therefore allowed us to incorporate ecological complexity whilst maintaining control over the genetic composition of the key antagonists.

MATERIALS AND METHODS

Host and parasite organisms

The host, *Daphnia magna* (Straus, 1820), is a freshwater crustacean that inhabits shallow freshwater ponds that are naturally susceptible to temperature fluctuations. The parasite, *Pasteuria ramosa* (Metchnikoff, 1888), is a spore-forming bacterium that sterilizes its hosts. *Daphnia magna* (hereafter: *Daphnia*) and *Pasteuria ramosa* (hereafter: *Pasteuria*) are a naturally coevolving host-parasite system (Decaestecker *et al.*, 2007). *Daphnia* commonly encounter *Pasteuria* transmission spores when filter feeding; once inside the host, spores cross the gut epithelium (Duneau *et al.*, 2011; Auld *et al.*, 2012) and proliferate (Auld *et al.*, 2014a), stealing resources that would otherwise be used for host reproduction (Cressler *et al.*, 2014). Millions of *Pasteuria* transmission spores are then released into the environment upon host death (Ebert *et*

115 *al.*, 1996). *Pasteuria* infection is easily diagnosed by eye: infected *Daphnia* have
116 obvious red-brown bacterial growth in their hemolymph, lack developed ovaries or
117 offspring in their brood chamber and sometimes exhibit gigantism (Ebert *et al.*, 1996;
118 Cressler *et al.*, 2014).

119 *Daphnia magna* are cyclically parthenogenetic: they reproduce asexually in
120 the main, but produce males and undergo sexual reproduction when environmental
121 conditions become unfavorable (Hobaek & Larsson, 1990). Host sex results in the
122 production of one or two eggs that are encased in an environmentally resistant
123 envelope called an ephippium. Once ephippia are released by the host, they fall to the
124 sediment and the eggs they contain hatch in later years. We collected three sediment
125 samples from Kaimes Farm, Leitholm, Scottish Borders, UK (2°20'43"W,
126 55°42'15"N) (Auld *et al.*, 2014b) in June 2014. From these sediment samples, we
127 isolated and hatched 21 sexually produced *Daphnia* resting eggs and propagated them
128 clonally by maintaining them under favorable conditions.

129

130 **Experimental setup**

131 We exposed ~20 *Daphnia* from each of the 21 *Daphnia* clonal lines to the original
132 sediment samples and isolated those hosts that became infected with *Pasteuria* (total
133 = 224 infected *Daphnia,* with a minimum of one infection per genotype). Each
134 infected *Daphnia* was individually homogenized and the density of *Pasteuria*
135 transmission spores was determined using a Neubauer (Improved) hemocytometer.
136 We then propagated these spores by exposing 5 x 10^5 *Pasteuria* spores from each
137 infected *Daphnia* to a further 80 healthy *Daphnia* of the same genotype (the
138 remaining spores were pooled and stored at -20°C). After 35 days, these *Daphnia*

were homogenized, pooled and the density of spores was determined. We then performed a second round of propagation. After three rounds of infection (isolation followed by two rounds of propagation), all spore samples were pooled and the total number was determined.

Meanwhile, we genotyped each of the 21 *Daphnia* clonal lines using 15 microsatellite loci (see *DNA extraction and microsatellite genotyping*), and selected the 12 most dissimilar multilocus genotypes for the mesocosm experiment. Replicate lines of each *Daphnia* of the 12 genotypes were maintained in a state of clonal reproduction for three generations to reduce variation due to maternal effects. There were five replicates per genotype; each replicate consisted of five *Daphnia* kept in 200mL of artificial medium ((Klüttgen *et al.*, 1994) modified using 5% of the recommended SeO_2 concentration (Ebert *et al.*, 1998)). Replicate jars were fed 5.0 ABS of *Chlorella vulgaris* algal cells per day (ABS is the optical absorbance of 650nm white light by the *Chlorella* culture). *Daphnia* medium was changed three times per week and three days prior to the start of the mesocosm experiment. On the day that the mesocosm experiment commenced, 1-3 day old offspring were pooled according to host genotype. Ten offspring per *Daphnia* genotype were randomly allocated to each of the 20 mesocosms (giving a total of 120 *Daphnia* per mesocosm).

Mesocosm experiment

Each mesocosm consisted of a 0.65m tall 1000 Liter PVC tank. Mesocosms were dug into the ground during July and August and were lined with ~10cm of topsoil; they were dug in to differing depths (0-0.64m) in order to promote variation in water temperature. The mesocosms were allowed to naturally fill with rainwater over an

eight month period prior to the experiment. During the experiment, half of the mesocosms experienced a weekly population mixing (physical flux) treatment, where mixed mesocosms were stirred once across the middle and once around the circumference with a 0.35m^2 paddle submerged halfway into the mesocosm (the exception to this was on the first day of the experiment, when all mesocosms experienced the mixing treatment to ensure hosts and parasites were distributed throughout the mesocosms). Deeper mesocosms had lower mean temperatures over the season (Spearman's Rank correlation: r_s = -0.98, p <0.0001). Mixing and temperature treatments were haphazardly distributed across the mesocosms, and mean temperature was not different between mixing treatments (mean temperature: t = 0.04, DF = 17.87, p = 0.97).

The experiment began on the 2nd April 2015 (Julian day 98), when 120 *Daphnia* (10 *Daphnia* x 12 genotypes) and 1 x 10^8 *Pasteuria* spores were added to each of the 20 mesocosms. Between the 2nd April and the 17th November 2015, we measured the temperature (°C, using an Aquaread AP-5000 probe; Aquaread, Broadstairs, Kent, UK) and depth of each mesocosm (m) on a weekly basis. After allowing a two week period for the *Daphnia* to establish (*i.e.*, from 16th April 2015), we measured the density of various *Daphnia* life stages in each mesocosm each week (juveniles, healthy adults, *Pasteuria*-infected adults). We did this by passing a 0.048 m^2 pond net across the diameter of the mesocosm (1.51 m) and counting the resulting *Daphnia*. If there were fewer than 100 *Daphnia* from the net sweep, we took a second sweep of the mesocosm. All *Daphnia* were returned to their respective mesocosms following population counts. Twenty-thirty *Daphnia* were sampled from each mesocosm for genotyping on two occasions during the season: once before peak epidemic (24th May 2015; Julian day 144) and once after peak epidemic (17th

November 2015; Julian Day 321). It is important to note that due to low population densities, we were only able to sample 16 of the 20 mesocosms (10 unmixed, 6 mixed) for population genetic analysis.

DNA extraction and microsatellite genotyping

Microsatellite genotyping was used to both identify the twelve unique multilocus *Daphnia* genotypes to follow their frequencies over the season during the experiment. We extracted genomic DNA from individual *Daphnia* using NucleoSpin Tissue XS (Macherey Nagel) following the manufacturers protocols. *Daphnia* were genotyped at 15 microsatellite markers assembled in two multiplexes for PCR reactions ((Jansen *et al.*, 2011); see Table S1 for a list of marker loci). For each reaction, forward primers were fluorescently labelled with different dyes, thus allowing us to identify four distinct loci. Multiplex PCR reactions were 10 µL in volume and consisted of 1µL DNA extract, 5µL of, 2x Type-it Multiplex PCR Mastermix (Qiagen), 3µL Nuclease Free H_2O and 1µL of 10x primer mix solution (2 µM of each primer). PCR Reactions were performed using the following protocol: Taq activation step at 95°C for 15 mins, followed by 30 cycles of 94°C for 30 secs, 57°C for 90 secs, 72°C for 90secs, 72 °C for 90 secs and a final extension at 60°C for 30 mins. PCR products were analyzed using an ABI 3730XL DNA Analyzer with the GeneScan-500 LIZ size standard (Applied Biosystems). Allele sizes were scored, using Geneous v9.0.5 (Biomatters) and validated manually.

Analysis

Data were analyzed using R 3.0.2. Data and code will be archived on Dryad upon acceptance of the manuscript. We analyzed how parasite prevalence varied over time using a Generalized Additive Model (GAM) with a binomial error distribution. GAMs fit non-parametric smoothing functions to covariates in a model (in this case, Julian Day), and allow comparisons between trajectories of the response variable with respect to other factors without the need to fit particular functions to the data. We fitted four GAMs to the parasite prevalence data: all models included the volume of water sampled as a covariate and Julian Day as a non-parametric smoother; physical flux treatment and mean mesocosm temperature were either fitted as fixed effects or as modifiers to the Julian Day smoother function in the other three models (see Table 1). We then compared the fits of the models using AIC in order to determine if the relationship between parasite prevalence and Julian Day varied according to mixing treatment, mean mesocosm temperature or both (Table 1). Since parasite prevalence depends on both the numbers of healthy and infected hosts, we fitted separate sets of GAMs with negative binomial errors to counts of infected and healthy adults in order to determine if mixing treatment or mean mesocosm temperature differentially affected hosts from different infection classes over time (see Table S2, S3). We also tested the relationship between epidemic size and severity. We did this by fitting a Generalized Linear Mixed Effects Model (GLMM) with binomial errors to data for the proportion of juveniles in the host population (a key measure of population of health given that the parasite sterilizes its host), with parasite prevalence and volume of water samples as fixed effects and host population and sample date fitted as random effects.

Second, we calculated the overall epidemic size for each mesocosm. This was done by integrating the area under the time series of empirically determined

prevalence for each mesocosm. We then tested how mean and variance in temperatures, and mixing treatment, affected overall epidemic size. This was done by fitting a linear model (LM) to the epidemic size data with mixing treatment, mean temperature, variance in temperature and all two-way interactions as fixed effects.

Third, we analyzed how host genotype frequencies changed over the course of the season. We analyzed mixed and unmixed mesocosms separately, using two LMs. For each LM, we fitted multilocus genotype identity and sampling time (start, pre-epidemic or post-epidemic) as fixed factors. We then performed *post hoc* tests to examine how genotype frequencies changed between the start and pre-epidemic sampling and between the pre-epidemic and post-epidemic sampling. In order to assess the level of genetic drift, we determined the level of among-population differentiation within mixing treatments and over time. We did this by calculating F_{ST} values for genotype data collected from mixed and unmixed mesocosms both pre- and post-epidemic. F_{ST} is a reliable measure of drift here, because we can be confident that standing host population consists of only asexually produced progeny (sexually produced eggs drop to the sediment and hatch in future years, and we found no recombinant genotypes in individuals collected throughout the experiment). Finally, we examined how host genotypic evenness (a measure of genetic diversity (Smith & Wilson, 1996)) covaried with mesocosm epidemic size and mixing treatment. We analyzed evenness data using a LM, with epidemic size, mixing treatment, sample time (pre- or post-epidemic) and all two-way interactions fitted as fixed factors.

RESULTS

Temperature and population mixing determine epidemic size

Pasteuria –infected hosts were observed from mid-May until mid-November (between Julian days 106 and 321). The timing and magnitude of *Pasteuria* epidemics varied across populations, as did various other environmental variables. Populations typically experienced a small peak in parasite prevalence in early June (~ day 160) and a much larger peak late July-early August (~day 210-250; Fig. 1). Both prevalence peaks were higher in unmixed than in mixed populations and the second peak was earlier and larger in warmer populations than in cooler ones (Fig. 1; Table 1). The shape of the relationship between parasite prevalence and time depended on both mixing treatment and mean temperature of the population (Fig. 1; Table 1). Further analysis revealed that warmer populations had higher numbers of infected hosts, but not healthy hosts, and that unmixed populations had higher numbers of both healthy and infected hosts than mixed populations (Table S2, S3, Fig. S1, S2). The proportion of the host population that consisted of juveniles was negatively associated with parasite prevalence (Fig. S3; GLMM: $z = 5.47$, $P < 0.0001$), demonstrating the impact of this sterilizing parasite on host populations. Overall epidemic size (measured as parasite prevalence integrated over time) was larger in populations where mean temperature was high (Fig. 2A; LM: $F_{1,16} = 8.70$, $P = 0.009$), variance in temperature was low (Fig. 2B; LM: $F_{1,16} = 4.52$, $P = 0.049$) and in populations that were unmixed (Fig. 2C; LM: $F_{1,16} = 8.81$, $P = 0.009$).

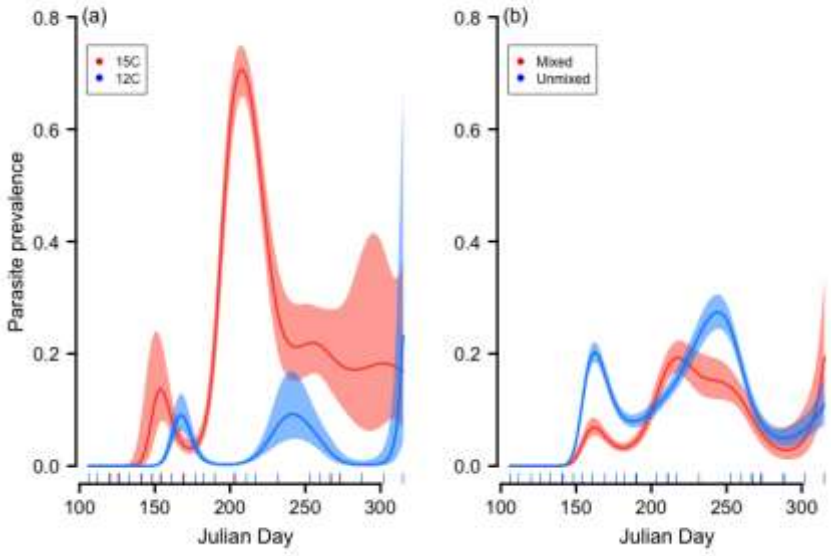

Figure 1. Parasite prevalence over time across 20 replicate mesocosm populations according to (a) mean population temperature and (b) population mixing treatment. The lines represent proportion of hosts infected as predicted by a generalized additive model (GAM; see Table 1) at ambient temperatures of 12°C and 15°C or for each mixing treatment (temperature was fitted as a covariate, but model predictions for two temperatures are shown for clarity). The shaded areas denote 95% confidence intervals (CIs).

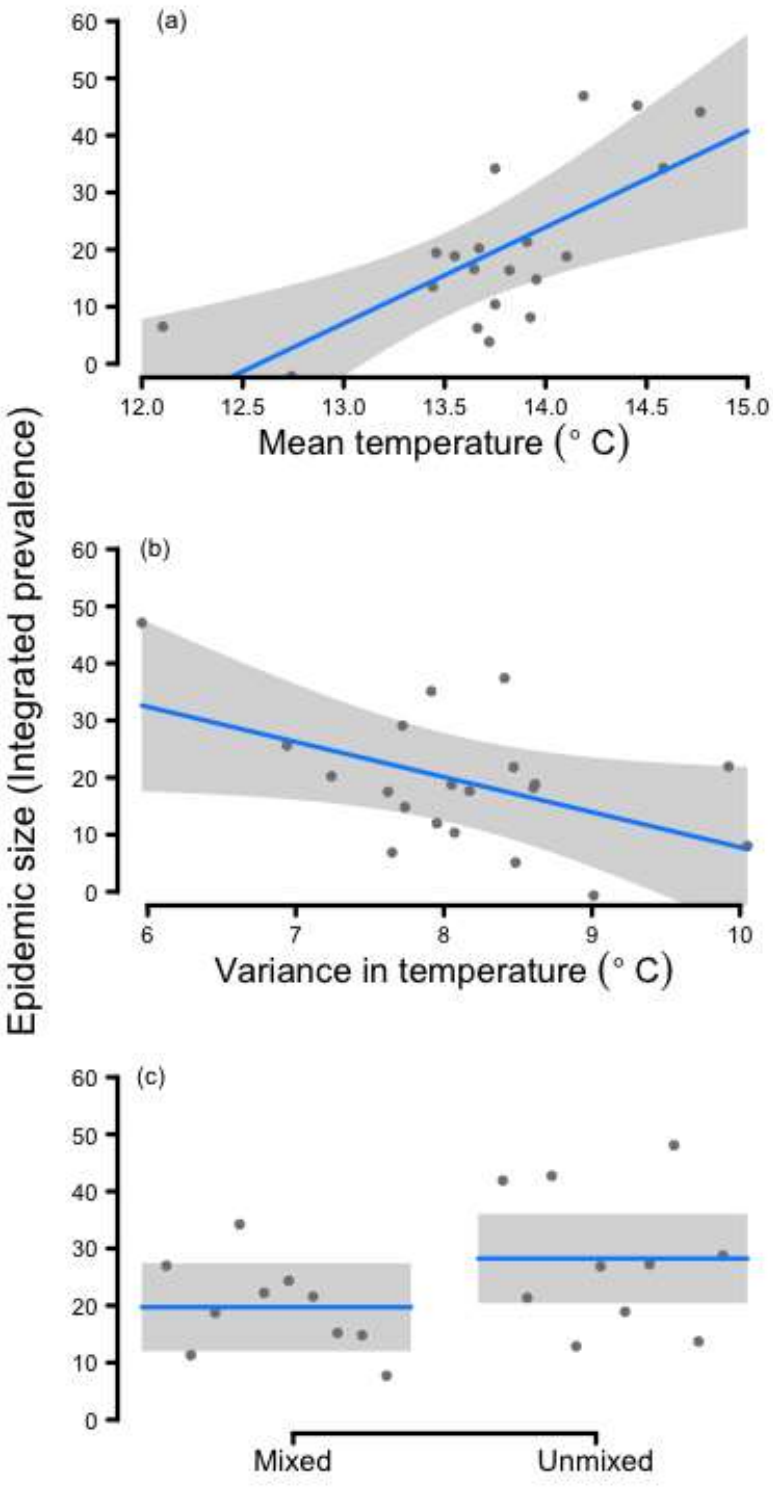

Figure 2. Relationship between epidemic size and (a) mean population temperature, (b) variance in population temperature, and (c) population mixing treatment. Lines show epidemic sizes as predicted by a linear model, shaded areas denote 95% CIs.

Table 1. **A** Generalized Additive Models fitted to parasite prevalence data. In all four models, sweep volume is fitted as a fixed effect and Julian Day as a non-parametric smoother; mean mesocosm temperature and mixing treatment are fitted as either fixed effects or as modifiers of the Julian Day smoother function. The model that best explains variation in parasite prevalence (here, the model with the lowest AIC value, model 4) is highlighted in bold. **B** Summary analysis for model 4. eDF is the estimated degrees of freedom.

A Model selection

Model	Parametric	Smoother	Deviance explained %	AIC
1	Sweep Vol; Mean Temp; Mixing	Julian Day	40.3	4754
2	Sweep Vol; Mean Temp	Julian Day by Mixing	40.4	4748
3	Sweep Vol; Mixing	Julian Day by Mean Temp	42.1	4647
4	**Sweep Vol**	**Julian Day by Mixing; Julian Day by Mean Temp**	**45.6**	**4428**

B Model 4 results

Response	Parametric/Smoother	Term	DF (eDF)	$\chi 2$	*P*
Parasite prevalence	Parametric	Sweep Vol	1	18.82	**<0.0001**
	Smoother	Julian Day by Mean Temp	9.77	477	**<0.0001**
	Smoother	Julian Day, Mixed	8.55	210.6	**<0.0001**
	Smoother	Julian Day, Unmixed	8.08	224.6	**<0.0001**

Epidemic size and population mixing shape host evolution

The relative frequencies of host genotypes changed over the course of the season, and the nature of this change clearly depended on both epidemic size and mixing treatment (Fig. 3). In unmixed mesocosms, genotype frequencies depended on an interaction between the identity of the genotype and the time of sampling (*i.e.*, whether the hosts were sampled at the start of the experiment, before the epidemic or after the epidemic. Fig. 4, LM: $F_{22,324} = 2.36$, $P = 0.0007$). *Post hoc* analysis revealed

that in unmixed mesocosms, genotype frequencies did not significantly change between the start of the experiment and when the pre-epidemic samples were taken (Tukey test: difference = -0.10, $P = 0.17$), but did change between the pre-epidemic and post-epidemic sampling (Tukey test: difference = -0.18, $P = 0.008$). In mixed mesocosms, genotype frequencies also depended on an interaction between the identity of the genotype and the time of sampling (Fig. 3, LM: $F_{22,180} = 1.72$, $P = 0.030$). However, *post hoc* tests showed a significant change in genotype frequencies between the start of the experiment and pre-epidemic sampling (Tukey test: difference = -0.21, $P = 0.032$), but no difference between the pre-epidemic and post-epidemic sampling (Tukey test: difference = -0.17, $P = 0.108$). Population genetic differentiation (a measure of genetic drift) was relatively low in unmixed mesocosms both before peak epidemic ($F_{ST} = 0.09$) and after peak epidemic ($F_{ST} = 0.10$) when compared to wild populations of a much larger size (Vanoverbeke *et al.*, 2007). In mixed mesocosms, population genetic differentiation was higher before peak epidemic ($F_{ST} = 0.12$) and increased towards the end of the season once the epidemic was over ($F_{ST} = 0.20$).

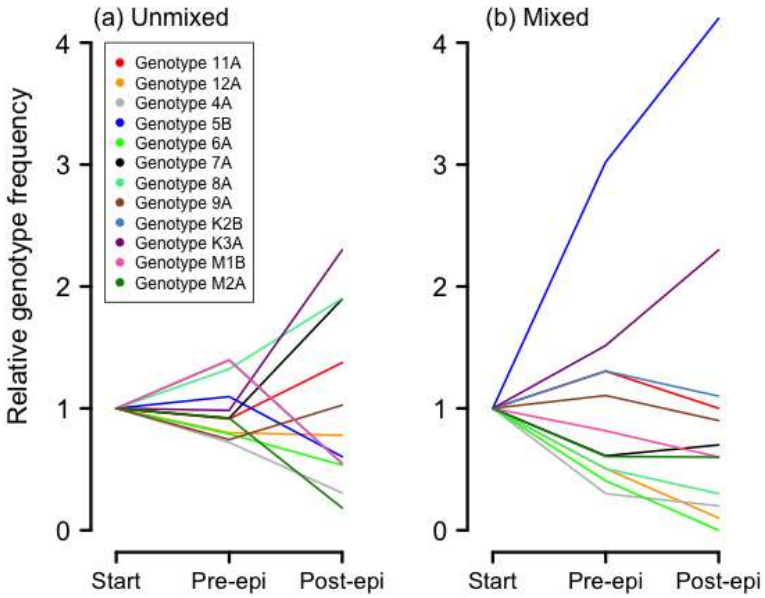

Figure 3. The relative frequencies of each genotype over time in (a) unmixed and (b) mixed populations. There are three sampling points: Start is the beginning of the experiment, when all genotypes were at the same frequency; Pre-epi was on May 24th 2015, before epidemics had peaked; and Post-epi was on November 17th 2015, after epidemics had peaked.

304

305 The relationship between genotypic evenness (a measure of host diversity) and
306 epidemic size depended on whether samples were collected before or after the
307 epidemic (Fig. 4, Table 2), where large epidemics were associated with low genotypic
308 evenness in samples collected after the epidemic had peaked (but not in samples
309 collected before peak epidemic). Genotypic evenness also depended on an interaction
310 between mixing treatment and sample time (Fig 4, Table 2): unmixed mesocosms had
311 higher genotypic evenness, especially in pre-epidemic samples.

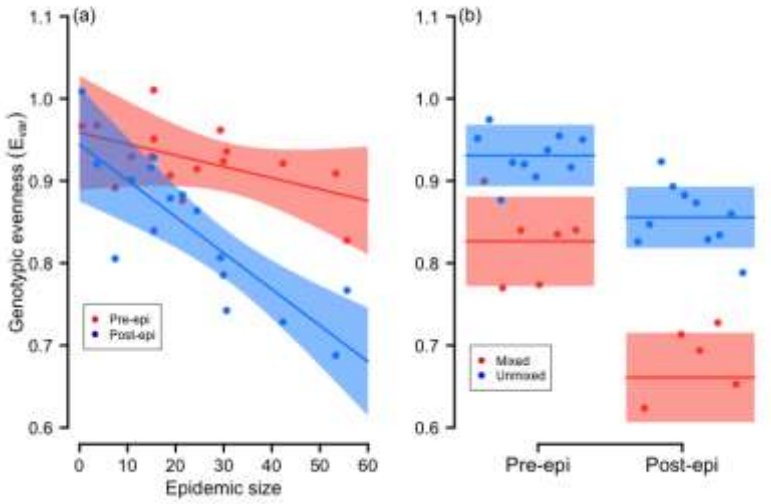

Figure 4. Relationship between host genotypic evenness and (a) epidemic size for pre-epidemic and post-epidemic samples, and (b) time of population sampling for mixed and unmixed populations. Lines show epidemic sizes as predicted by a linear model, shaded areas denote 95% CIs.

Table 2. Linear model testing effects of epidemic size, population mixing and sampling time (start, pre-epidemic, post-epidemic) on host genotypic evenness (E_{var}, a measure of host diversity)

Genotypic Evenness (E_{var})	DF	SS	*F*	*P*
Epidemic size (Epi)	1	0.036	16.77	**0.0004**
Mixing treatment (Mix)	1	0.113	52.62	**<0.0001**
Sampling time (Samp)	1	0.113	52.44	**<0.0001**
Epi x Mix	1	0.001	0.37	0.55
Epi x Samp	1	0.011	5.16	**0.032**
Mixed x Samp	1	0.009	4.32	**0.048**
Error	25	0.054		

DISCUSSION

Much of our understanding of how climate change affects disease either comes from controlled laboratory experiments, where the environmental effects can be effectively dissected but ecological realism is lacking, or from observational studies of populations, where ecological complexity can mask the drivers of variation in disease.

Semi-natural populations provide an excellent opportunity to manipulate environmental conditions while embracing ecological realism (Benton *et al.*, 2007), but see also (Paull & Johnson, 2014). We found that twenty *Daphnia* populations - each consisting of an identical suite of twelve genotypes - suffered very different epidemics of the sterilizing parasite *Pasteuria ramosa* depending on the temperature and mixing treatments they experienced. However, whilst epidemics differed among mesocosm populations, they were similar to natural epidemics in wild populations in that they occurred in the summer and ended in the winter. Both the timing and magnitude of epidemics and the strength of parasite-mediated selection was dependent on mean temperature, temperature variability and population mixing. Furthermore, the mode and tempo of host evolution, and thus the genetic diversity of host populations, was shaped by both epidemic size and mixing treatment.

In numerous host-parasite systems, warmer temperatures are associated with increased parasite transmission, within-host growth rates, or both (LaPointe *et al.*, 2010; Alonso *et al.*, 2011; Baker-Austin *et al.*, 2013; Burge *et al.*, 2013; Elderd & Reilly, 2014), though see also (Raffel *et al.*, 2013). Laboratory experiments using the *Daphnia-Pasteuria* system demonstrated increased likelihood of infection, higher parasite burdens and increased host mortality rates when hosts were incubated at 20°C than at 15°C (Vale *et al.*, 2008; Vale & Little, 2009). However, those studies also demonstrated that warming led to increased fecundity in uninfected *Daphnia* (as is common in numerous organisms: Huey & Berrigan, 2001; Hochachka & Somero, 2016). This raises the question of whether the costs of infection are mitigated by the benefits of increased fitness in uninfected hosts in natural populations. We found that even small increases in ambient temperature (3°C) were associated with increased overall epidemic size. We also found that over the course of the season, warmer

mesocosms had greater numbers of juveniles and infected adults, but similar numbers of healthy adults. High prevalence of this sterilizing parasite was, however, associated with a low proportion of juveniles in the host population. Our data therefore suggest that any warming-induced increase in reproduction in healthy hosts served to fuel the epidemic more than growth of the healthy host population.

It is not just mean temperature that is important for disease dynamics; temperature variability also plays a major role. Daily temperature variation was found to be negatively associated with the likelihood of the Dengue virus successfully infecting its *Anopheles gambiae* (mosquito) hosts (Lambrechts *et al.*, 2011), and the *Holospora undulata* bacterium infecting its *Paramecium caudatum* hosts (Duncan *et al.*, 2011). Whereas, rapid temperature fluctuations increased the likelihood that the fungus *Batrachochytrium dendrobatidis* successfully infected its *Osteopilus septentrionalis* (frog) hosts (Raffel *et al.*, 2013) and also fostered greater *B. dendrobatidis* growth rate on *Notophthalmus viridescens* (newt) hosts (Raffel *et al.*, 2015). Although we did not measure daily temperature fluctuation, we did find that increased weekly temperature variability was associated with smaller *Pasteuria* epidemics. It is unclear exactly how temperature variability limits epidemics in this host-parasite system. However, parasite ability to attach to hosts is very temperature sensitive in the related bacterium, *Pasteuria penetrans*, a parasite of nematodes: a 7.5°C deviation from thermal optimum leads to a 15% reduction in *P. penetrans* attachment to the nematode cuticle (Freitas *et al.*, 1997), suggesting parasite attachment should be the focus of future study on how temperature variability affects infection in the *Daphnia magna-Pasteuria ramosa* system.

In addition to shifts in temperature, changing weather has given rise to

increased physical flux in the form of storms and floods. Such flux is known to cause increased mixing in populations and nutrient upwelling (Walker, 1991), with the potential to increase host contact rate with parasite transmission stages and thus epidemic size (May & Anderson, 1979). Based on this, one might expect mixing to lead to larger epidemics, though we found no evidence for this. Contrary to expectations, we found that mixed mesocosms suffered smaller epidemics. It is, however, important to note that population size was universally lower in mixed than in unmixed mesocosms, perhaps because sediment upwelling reduced the efficiency at which *Daphnia* filtered food from the water, thus leading to a lower carrying capacity. So if there were any increases in parasite infection rates due to higher host-parasite contact rate, they were outweighed by negative effects on host reproductive rate.

Given that each mesocosm was seeded with identical suites of host genotypes that reproduced asexually throughout the season, we were able to test whether any emergent patterns of selection were shaped by environmental variation and quantify the genetic drift in host populations. Directional selection would favor the same subset of host genotypes across populations, whereas if genetic drift was the principal driver of host evolution, we would observe relatively high among-population genetic differentiation (Vanoverbeke *et al.*, 2007; Vanoverbeke & De Meester, 2010). In unmixed mesocosms, we found that the frequencies of each genotype changed over the course of the season, and the nature of this change depended on the identity of the genotype. Importantly, there was no significant change in genotype frequencies between the start of the experiment and the sample taken before the peak epidemic, but there was a significant change in genotype frequencies between the pre-epidemic and post-epidemic sampling. Among unmixed mesocosms, population genetic

differentiation was low (given the small size of the populations: Vanoverbeke *et al.*, 2007) and changed minimally over the course of the season. A strong signal of parasite-mediated selection was therefore discernible over and above drift, supporting disease epidemics as the principal driver of host evolution in unmixed mesocosms.

Mixed mesocosms showed a different pattern. Whilst the direction of change in genotype frequencies also depended on the identity of the genotype in mixed mesocosms, the significant changes occurred before the peak epidemic. Furthermore, the two host genotypes that increased most in frequency (5B and K3A) were comparatively susceptible to the parasite but had the highest reproductive rates (S. Auld unpublished data). These results are consistent with our epidemiological data, and suggest that mixing exerts strong selection for high fecundity in the host population and that parasite epidemics play a less important role on host evolution than in unmixed mesocosms. On the other hand, population differentiation increased over the course of the season in mixed mesocosms, suggesting that mixing led to a bottleneck that left the host population particularly vulnerable to genetic drift.

We sought to test if parasite-mediated selection maintained host genetic diversity (Wolinska & Spaak, 2009) or depleted it by driving selective sweeps (Obbard *et al.*, 2011). Host genotypic evenness – a key measure of population genetic diversity - was negatively associated with epidemic size, particularly in samples collected after peak epidemic. This provides compelling evidence that parasite epidemics apply strong directional selection on host populations. Mixed mesocosms also had lower host genotypic evenness than unmixed populations; once again, this effect was stronger for samples collected after the peak epidemic, and points towards the mixing treatment stripping out host genetic diversity over time. How could this

affect the health of populations in the long-term? Selection for increased host resistance could lead to smaller or less severe epidemics in future years. If so, one would expect mesocosms that suffered the largest epidemics in this season to suffer smaller epidemics in the following year. However, this relies on the assumption that host genes that confer resistance to current parasites also confer resistance to future parasites (this is sometimes, though not always the case in this system: (Little & Ebert, 2001; Auld *et al.* 2016). In any case, host populations with low genetic diversity are commonly prone to the spread of severe epidemics because disease transmission is more likely to be successful when hosts are genetically similar (Anderson *et al.*, 1986; King & Lively, 2012). Moreover, a decline in genetic diversity reduces a population's capacity to respond to further selection more generally, because diversity is the currency with which a population pays for adaptation (Lande & Shannon, 1996). Therefore, the low diversity populations in mixed mesocosms are still much more vulnerable to extinction, despite suffering smaller parasite epidemics.

STATEMENT OF AUTHORSHIP

SKJRA designed the study, SKJRA and JB collected the data, SKJRA analyzed the data and wrote the first draft of the manuscript, and both authors approved the final version of the manuscript.

DATA ACCESSIBILITY STATEMENT

All data and code will be archived with Dryad upon acceptance of the manuscript.

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Figure 1. Parasite prevalence over time across 20 replicate mesocosm populations according to (a) mean population temperature and (b) population mixing treatment. The lines represent proportion of hosts infected as predicted by a generalized additive model (GAM; see Table 1) at ambient temperatures of 12°C and 15°C or for each mixing treatment (temperature was fitted as a covariate, but model predictions for two temperatures are shown for clarity). The shaded areas denote 95% confidence intervals (CIs).

Figure 2. Relationship between epidemic size and (a) mean population temperature, (b) variance in population temperature, and (c) population mixing treatment. Lines show epidemic sizes as predicted by a linear model, shaded areas denote 95% CIs.

Figure 3. The relative frequencies of each genotype over time in (a) unmixed and (b) mixed populations. There are three sampling points: Start is the beginning of the experiment, when all genotypes were at the same frequency; Pre-epi was on May 24th 2015, before epidemics had peaked; and Post-epi was on November 17th 2015, after epidemics had peaked.

Figure 4. Relationship between host genotypic evenness and (a) epidemic size for pre-epidemic and post-epidemic samples, and (b) time of population sampling for mixed and unmixed populations. Lines show epidemic sizes as predicted by a linear model, shaded areas denote 95% CIs.

Table 1. **A** Generalized Additive Models fitted to parasite prevalence data. In all four models, sweep volume is fitted as a fixed effect and Julian Day as a non-parametric smoother; mean mesocosm temperature and mixing treatment are fitted as either fixed effects or as modifiers of the Julian Day smoother function. The model that best explains variation in parasite prevalence (here, the model with the lowest AIC value, model 4) is highlighted in bold. **B** Summary analysis for model 4. eDF is the estimated degrees of freedom.

Table 2. Linear model testing effects of epidemic size, population mixing and sampling time (start, pre-epidemic, post-epidemic) on host genotypic evenness (E_{var}, a measure of host diversity).