

26 **One sentence summary:** Ecological factors affect host-parasite coevolution.

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29 Parasites are a strong selective force acting on host populations, and *vice versa*^{1,2},
30 fuelling rapid cycles of adaptation and counter-adaptation in terms of host resistance
31 and parasite capacity to infect²⁻⁵. These coevolutionary processes can have profound
32 effects on disease outbreaks. For example, whether the host or the parasite is ahead in
33 the coevolutionary process can, in part, affect whether epidemics are emerging⁶ or in
34 decline⁷. A key aim of evolutionary ecologists is to understand the extent to which
35 coevolution is: (1) a deterministic process with repeated, predictable outcomes that
36 are either hard-wired or shaped by measurable abiotic and biotic ecological variation;
37 and (2) a stochastic process driven by unpredictable events.

38 Ecological variation is known to have strong effects on coevolution⁸⁻¹⁰. However,
39 dissecting host-parasite coevolution in biologically realistic settings is fraught with
40 difficulty, and much of our understanding of coevolution therefore comes from
41 laboratory experiments that eliminate ecological complexity. This experimental
42 control comes at a cost to biological realism, because parasitism is just one of many
43 ecological interactions that hosts experience in the wild; predation, competition *etc.*,
44 and abiotic variables such as temperature are already known to either amplify or
45 diminish host evolutionary responses to parasite-mediated selection^{4,11-15}. By contrast,
46 we expect parasite evolution, particularly for obligate endoparasites, to be driven
47 primarily by shifts in host-mediated selection caused by changes in host genotype
48 frequencies¹⁶, because hosts insulate their endoparasites from the wider environment.
49 These asymmetries in host and parasite responses to reciprocal selection could create
50 discrepancies between coevolution observed in the laboratory and in the natural arena.

51 We quantified how coevolutionary trajectories varied among 16 biologically realistic
52 pond populations of *Daphnia magna* and its sterilizing bacterial endoparasite,
53 *Pasteuria ramosa*. Each pond was initiated with an identical suite of *Daphnia*
54 genotypes and the same starting population and dose of *Pasteuria* transmission
55 spores, and the densities of healthy and parasite-infected were then monitored weekly
56 over the course of each pond epidemic. At the end of the epidemic, *Daphnia* were
57 sampled to determine the change in genotype frequencies and additional infected
58 *Daphnia* were sampled to obtain parasite isolates from each pond. We subsequently
59 conducted a time-shift experiment where we exposed replicates of the original twelve
60 *Daphnia* genotypes to either the ancestral parasite used to initiate the pond
61 populations, or to parasite isolates collected from each pond at the end of the
62 epidemic.

63 By combining data from the time-shift experiment with changes in relative genotype
64 frequencies, we dissected, for each pond, the effects of the three components of host-
65 parasite coevolution on the change in parasite transmission rate over the course of the
66 season: host evolution of resistance, parasite evolution of infectivity, and coevolution
67 (*i.e.*, the extent to which the parasite population non-additively evolved in response to
68 a changed complement of host genotypes). When host genotypes that were resistant to
69 the ancestral parasite increased in frequency within a population, that host population
70 evolved host resistance; when a parasite sample collected at the end of the season
71 caused more infections than the ancestral parasite when exposed to the panel of host
72 genotypes, that parasite population evolved increased infectivity; and when a parasite
73 sample collected at the end of the season became proportionately more infectious to
74 host genotypes that were resistant to the ancestral parasite, that parasite population
75 coevolved in response to the changing complement of host genotypes.

76 **Results and Discussion**

77 **Coevolutionary trajectories varied among ponds.** Whilst the ponds had the same
78 starting populations of hosts and parasites, each pond experienced its own natural
79 temperature profile (with significant variation across ponds), and half underwent an
80 experimental manipulation of within-population flux (mixing) that simulated extreme
81 precipitation events. We recorded the natural variation in 10 biotic and abiotic
82 ecological variables over the season: temperature, pH, dissolved oxygen, chlorophyll,
83 nitrate, and total dissolved salt, parasite prevalence, predator density and adult host
84 density. This allowed us to examine the role of ecological variation early in the season
85 in driving coevolutionary divergence.

86 We found that each pond population followed its own coevolutionary trajectory (with
87 respect to changes in parasite transmission rate). This was driven by variation in all
88 three coevolutionary axes: host evolution, parasite evolution and coevolution (Fig. 1a-
89 c). We uncovered asymmetry in the magnitude of host and parasite evolution: parasite
90 populations evolved more in their capacity to infect the ancestral host population than
91 their corresponding hosts evolved capacity to resist the ancestral parasite population
92 (paired $t = -3.25$, $P = 0.005$; Fig. 1). We also found a strong positive relationship
93 between the change in host resistance and coevolution, *i.e.*, a change in transmission
94 rates due to a shifting complement of host genotypes ($r_s = 0.69$, $P = 0.004$; Fig. 1b):
95 over the course of the season, parasites became disproportionately better at infecting
96 those host genotypes that were previously resistant at the beginning of the season
97 (host genotypes that had become more common), and also disproportionately poorer
98 at infecting host genotypes that were previously susceptible at the beginning of the
99 season (host genotypes that had become rarer). By contrast, there was a lack of
100 relationship between the change in parasite infectivity and coevolution ($r_s = 0.39$, $P =$

101 0.135; Fig. 1c). These findings are consistent with the idea that ecological interactions
102 above and beyond parasitism can select on hosts, but do not act on the host insulated
103 parasites; shifts in host genotype frequencies instead drive parasite genetic change *via*
104 coevolution. Whereas, for ectoparasites, which live on the host exterior, wider
105 ecological conditions are known to shape the evolution of virulence^{17,18}.

106 **Ecology drives variation in coevolution.** Initial inspection of the ten ecological
107 variables in isolation revealed that the mixing treatment had no effect on nine of the
108 ten ecological variables, but that it was associated with lower total adult host densities
109 (see Table S1). This supports the idea that the mixing treatment affected the ecology
110 of the system primarily by reducing host densities directly; indeed, it is known that
111 sediment suspension can interfere with *Daphnia* filter feeding, reducing population
112 growth and the consumption of algae¹⁹ (see later results). Higher temperatures and
113 lower chlorophyll concentration, dissolved oxygen and pH were each associated with
114 the evolution of host resistance, but none of the ecological variables were associated
115 with parasite evolution or coevolution (see Table S2).

116 However, a more holistic multivariate analysis uncovered a much more interesting
117 story. A Principal Components Analysis of the biotic and abiotic variables (Fig. S1)
118 revealed considerable ecological variation among populations, with the first and
119 second PC axes explaining 36.0% and 21.6% of that variation. The main factors
120 driving variation in unmixed populations were mean temperature and host density,
121 whereas several factors explained variation in mixed populations: chlorophyll,
122 predator density, oxygen, pH and nitrate. There was a strong positive relationship
123 between δ_{eco} the pairwise Mahalanobian distances between populations in
124 multivariate space for ecological variation, and δ_{coevo} , the pairwise Mahalanobian
125 distances for coevolutionary net change (Fig. 2: Mantel $r = 0.36$, $P = 0.029$).

126 Populations that were more ecologically different from each other had more divergent
127 coevolutionary trajectories. Both theory²⁰ and empirical data (reviewed in¹⁰) have
128 previously shown how host and parasite genotypes can differentially respond to
129 particular environmental variation to create (co)evolutionary hotspots and coldspots²¹;
130 these results show how such environmental variables can act in concert to mediate
131 coevolution.

132 **Ecology affects host evolution, with consequences for coevolution.** The next step
133 was to dissect precisely how ecological variation and coevolutionary change were
134 linked. Using Structural Equation Modelling (SEM; Fig. S3), we tested which of two
135 credible scenarios better explained the relationship between ecological and
136 coevolutionary variation among populations (Fig. 3). Scenario 1 (SEM1) proposed
137 that mixing affected ecology (measured as PC1), that ecology directly affected host
138 evolution, parasite evolution and coevolution, and that parasite evolution also
139 separately affected coevolution. Scenario 2 (SEM2) was similar, except it proposed
140 that ecology did not affect coevolution directly; here ecological effects on coevolution
141 were mediated by both host evolution and parasite evolution (see methods section for
142 details). Whilst both SEM1 and SEM2 both provided adequate fit to the data (SEM1:
143 Fisher's $C = 19.80$, D.F. = 12, $P = 0.071$, BIC = 64.16; SEM2: Fisher's $C = 12.66$,
144 D.F. = 12, $P = 0.394$, BIC = 57.02), SEM2 was the better performing model (Δ BIC =
145 7.14), demonstrating that there was greater support for the scenario where ecological
146 effects on coevolution were mediated by both host evolution and parasite evolution.

147 Analysis of SEM2 revealed that ecological conditions, as expressed by PC1, were
148 significantly different between mixed and unmixed populations (Fig. 3; Fig. 4a; Table
149 S3), and that epidemic size was negatively associated with this measure of ecological
150 variation (Fig. 4b; Table S3), such that epidemics were larger in populations that were

151 warmer, had lower chlorophyll concentrations, lower pH and lower predator densities.
152 Epidemic size was associated with the evolution of host resistance (reduced
153 transmission rate) (Fig. 4c; Table S3), but there was no compelling evidence for an
154 association between epidemic size and parasite infectivity (Fig. 4d; Table S3), or
155 coevolution (Fig. 4e; Table S3). Ecology was also directly associated with evolution
156 of host resistance (Fig. 4f; Table S3), but not parasite infectivity (Fig. 4g; Table S3).
157 Finally, the ability to examine partial residuals after controlling for other variables (a
158 major advantage of the SEM approach) allowed us to uncover that coevolution was
159 positively associated with both the evolution of host resistance (Fig. 4h; Table S3) and
160 the evolution of parasite infectivity (Fig. 4i; Table S3).

161 These separate effects of epidemic size and wider ecology on host (but not parasite)
162 evolution provide two principal insights. They add support our assertion that hosts are
163 subject to a wide range of selective pressures due to both parasite-mediated selection
164 from disease epidemics and from wider ecology, whereas the parasite's insulation
165 within the host environment and the obligate nature of its relationship with the host
166 ensures the host is the principal agent of selection (hence the relationship between
167 host evolution and coevolution). They also raise the intriguing hypothesis that
168 epidemic size and wider ecology (driven in part by mixing treatment) pull two
169 separate levers to drive host evolution of resistance. First, larger epidemics could have
170 exerted greater parasite-mediated selection for host resistance¹³. Second, populations
171 with greater PC1 values, *i.e.*, lower predation and higher temperatures (and thus
172 higher *Daphnia* reproductive rate), had high population densities^{22,23}, and therefore
173 likely had a greater capacity to respond to any parasite-mediated selection. This may
174 have fuelled coevolution, driving the divergence in coevolutionary trajectories we see
175 in Fig 1.

176 The next step is to explain the relationships between host evolution, parasite evolution
177 and coevolution. Previous work demonstrated the Matching Allele Model (MAM)
178 best describes the infection genetics of the *Daphnia-Pasteuria* system^{4,24,25}: alleles
179 conferring parasite ability to infect one host genotype often preclude it from infecting
180 other different host genotypes¹⁴. However, MAM in its purest sense requires just one
181 susceptible host genotype for every infectious parasite genotype²⁶, but in the
182 *Daphnia-Pasteuria* system, parasite genotypes commonly infect >1 host genotypes
183 and also vary in the number of host genotypes each parasite can infect²⁷. This
184 deviation from MAM could potentially explain why coevolution was positively
185 associated with the evolution of host resistance and, to a lesser extent, parasite
186 infectivity (Fig. 4h,i; Table S3): parasite populations that were more infectious to the
187 ancestral complement of hosts were also better at infecting the new complement of
188 hosts, and hosts that got better at resisting the ancestral parasite also got better at
189 resisting the evolved parasite. Reciprocal selection could have acted in two ways.
190 First, general selection could have favoured parasite genotypes that infect the broadest
191 range of host genotypes (and *vice versa* for resistance in host genotypes), and second,
192 specific selection could have separately favoured parasite genotypes that could infect
193 host genotypes that had become particularly common (again, *vice versa* for resistance
194 in hosts genotypes).

195 **Conclusion**

196 These results demonstrate that even in seemingly noisy environments, coevolution
197 was still largely driven by deterministic, ecologically-mediated processes. Individual
198 biotic and abiotic variables gave us a small glimpse of how wider ecology shaped
199 coevolution. It was only after viewing multiple ecological variables from a
200 multivariate perspective that we were able to observe that the ecological theatre

201 determined the (co)evolutionary play in a measurable understandable way (*sensu*²⁸).
202 Recent work has demonstrated that quantitative differences among qualitatively
203 similar environments can explain evolutionary divergence among stickleback
204 populations²⁹; we show the same is true for more complex host-parasite coevolution,
205 and that knowledge of multiple ecological conditions could help us predict the
206 distribution of coevolutionary hotspots and coldspots²¹.

207

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218 **References**

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296

297 **METHODS**

298 **Pond experiment.** The pond experiment was used to test how epidemic size varied
299 across populations that were initiated with the same suite of hosts and parasites, but
300 experienced biologically realistic variation in biotic and abiotic ecological variables.
301 Additionally, healthy and infected hosts were sampled at the end of the season in
302 order to quantify the change in relative host genotype frequencies across populations
303 and provide parasite samples for the time shift experiment.

304 To start with, replicate lines of the 12 genotypes of *Daphnia magna* were maintained
305 in the laboratory in a state of clonal reproduction for three generations to reduce
306 variation due to maternal effects. There were five replicates per genotype; each
307 replicate consisted of five *Daphnia* kept in 200 mL of artificial medium³⁰ modified
308 using 5% of the recommended SeO₂ concentration³¹. Replicate jars were fed 5.0 ABS
309 of *Chlorella vulgaris* algal cells per day (where ABS is the optical absorbance of 650
310 nm white light by the *Chlorella* culture). *Daphnia* medium was changed three times
311 per week and three days prior to the start of the pond experiment. On the day that the
312 pond experiment commenced, 1–3 day old offspring were pooled according to host
313 genotype. Ten offspring per genotype were randomly allocated to each of the 16
314 ponds (giving a total of 120 *Daphnia* per pond). From preliminary work, we knew
315 that the 12 genotypes used in our pond and laboratory experiments were a
316 representative sample of parasite resistance profiles observed in the source
317 population. The proportion of *Daphnia* that became infected with the ancestral

318 mastermix *Pasteuria* after 48h exposure to 2×10^5 spores ranged from 0 to 0.75
319 depending on genotype, with a mean of 0.27.

320 Each pond consisted of a 0.65 m tall 1000 Liter PVC tank filled with rainwater. The
321 ponds were set to different depths into the ground and experienced different
322 temperature profiles³². In addition, six of the ponds experienced a weekly mixing
323 treatment where mixed ponds were stirred once across the middle and once around the
324 circumference with a 0.35 m² paddle submerged halfway into the pond (the exception
325 to this was on the first day of the experiment, when all ponds experienced the mixing
326 treatment to ensure hosts and parasites were distributed throughout the ponds).

327 The experimental coevolution began on the 2nd April 2015 (Julian day 98), when 120
328 *Daphnia* (10 *Daphnia* x 12 genotypes) and 1×10^8 *Pasteuria* spores from the
329 ancestral mastermix were added to each of the 16 ponds. The ancestral mastermix
330 comprised *Pasteuria ramosa* spores propagated using 21 separate *Daphnia* genotypes
331 exposed to sediment from their original pond (Kaimes, Scottish Borders, UK³²).
332 Between the 2nd April and the 17th November 2015, we measured key abiotic and
333 biotic ecological variables on a weekly basis. Temperature, pH, dissolved oxygen
334 (%), chlorophyll ($\mu\text{g. L}^{-1}$), nitrate (mg.L^{-1}) and total dissolved salt (mg.L^{-1}) were
335 recorded using an Aquaread AP-5000 probe (Aquaread, Broadstairs, Kent, UK). Host
336 density (L^{-1}), parasite prevalence and predator density (L^{-1}) were determined using
337 standard sampling procedures³².

338 Twenty-thirty *Daphnia* were sampled from each pond for genotyping after peak
339 epidemic (17th November 2015; Julian Day 321). The DNA extraction and
340 microsatellite genotyping process is described in full in¹⁴. Microsatellite genotyping

341 was used to identify the twelve unique multilocus *Daphnia*, and thus track the change
342 in relative genotype frequencies between the beginning of the experiment (when all
343 genotypes were at equal frequencies) and the end of the experiment. The relative
344 genotype frequencies were used as a measure of relative genotype fitness within each
345 pond. Finally, we sampled 90 infected hosts from each of the 16 ponds, which were
346 homogenised and pooled into three replicate isolates per pond (30 infected *Daphnia*
347 per isolate).

348 **Time shift experiment.** The time shift experiment was used to understand host and
349 parasite evolution over the course of the epidemic. Specifically, the same panel of
350 host genotypes used to initiate the pond populations was exposed to either the
351 ancestral parasite, or to parasite samples collected from each population at the end of
352 the epidemic, following a fully factorial design.

353 We established maternal lines for each of the 12 *Daphnia* genotypes used in the pond
354 experiment. There were three replicates per genotype; each replicate consisted of
355 eight adult animals in 100ml of artificial media. The *Daphnia* were fed 0.5 ABS
356 chemostat-grown *Chlorella vulgaris* algae per *Daphnia* per day. Jars were incubated
357 at 20°C on a 12L:12D light cycle, and their media was changed three times per week.
358 Offspring from early instars were taken from the second brood for use in the time
359 shift assay.

360 The experimental design consisted of a factorial manipulation of the 12 host
361 genotypes and parasite samples collected from each pond ($n = 16$) plus the original
362 (ancestral) parasite mixed isolate used to seed the populations. There were three
363 independent replicate parasite isolates collected from each pond and a further three
364 replicate isolates of the ancestral parasite (17 parasite treatments; three replicates per

365 treatment). On the day of treatment exposure, neonates from each maternal line were
366 assigned to experimental jars (8 per jar, in 100ml of artificial media) and allocated to
367 parasite treatments following a split-clutch design. There was a total of 612
368 experimental jars (4896 *Daphnia*). Each jar received a dose of 2×10^5 *Pasteuria*
369 spores and kept under identical conditions as the maternal lines. After 48 hours
370 exposure to the *Pasteuria* spores, the experimental *Daphnia* were transferred into
371 fresh media. The infection status of each *Daphnia* was determined by eye 25 days
372 post exposure.

373 Using the results of these infection experiments for each host-parasite combination,
374 we calculated transmission rate (β , L spore⁻¹ day⁻¹) using the following equation:

$$\beta = -\frac{1}{Z_0 \cdot t} \cdot \ln\left(\frac{S_t}{S_0}\right) \quad (1)$$

375

376 where Z_0 is the starting density of spores, t is the duration of the trial
377 exposure, S_t is the density of uninfected hosts at the end of the exposure and S_0 is the
378 initial density of hosts.

379 **Dissection of host-parasite (co)evolution.** By combining transmission rate data from
380 the time shift experiment with relative genotype frequency data from the pond
381 experiment, we dissected the various host and parasite contributions towards the
382 evolution of transmission rate.

383 To achieve this, we calculated the change in parasite transmission rate over the course
384 of the season and its three contributory components (eq. 2): change in parasite
385 transmission rate due to evolution of host resistance to the ancestral parasite
386 (hereafter, change in host resistance, $\Delta\beta_h$), change in parasite transmission rate due to

387 evolution of parasite infectivity to a set of reference hosts (hereafter, change in
388 parasite infectivity, $\Delta\beta_p$), change in parasite transmission rate due to evolution of
389 parasite infectivity to the evolved host population (non-additive coevolution and
390 hereafter, coevolution, $\Delta\beta_{hp}$).

$$\Delta\beta = \Delta\beta_h + \Delta\beta_p + \Delta\beta_{hp} \quad (2)$$

391 We used two essential pieces of information to determine how host evolution, parasite
392 evolution and coevolution contributed to changes in overall transmission rate for each
393 population: the change in the relative frequency of each host genotype within each
394 population during the course of the pond experiment; and the difference in the
395 susceptibility of these genotypes relative to the ancestral parasite mix used to seed the
396 populations and the parasite samples collected at the end of the epidemic.

397 First, we calculated the relative frequency of each genotype within each pond at the
398 end of the epidemic. This was done as follows:

$$\bar{w}_{h,t} = P_{h,t} \cdot n_h \quad (3)$$

399 where $P_{h,t}$ is the frequency of host genotype h at time t , and n_h is the total
400 number of host genotypes used to seed the population (in this case, $n_h = 12$). The
401 coevolution experiment started at $t = 0$, when all hosts had a genotype frequency of
402 1, and ended at $t = 1$.

403 Then for each population, we calculated the overall change in mean transmission rate.
404 This was done by determining the change in parasite transmission rate for each host
405 genotype between the end of epidemic parasite samples and the ancestral parasite
406 sample, and weighting by the change in host genotype frequency to calculate a mean
407 for each population:

408
$$\Delta\beta = \frac{1}{n_h} \cdot \sum_h \left((\beta_{h,t=1} \cdot \bar{w}_{h,t=1}) - \beta_{h,t=0} \right), \quad (4)$$

409 where $\beta_{h,t}$ is the transmission rate of each host genotype.

410 Next, we calculated the mean change in transmission rate due to population-level
 411 evolution of host resistance to the ancestral parasite ($\Delta\beta_h$) by calculating the mean
 412 resistance to the ancestral parasite weighted by the change in host relative genotype
 413 frequency for each population (eq. 5) and the mean change in transmission rate due to
 414 parasite evolution in the capacity to infect the ancestral host population ($\Delta\beta_p$, eq. 6).

$$\Delta\beta_h = \frac{1}{n_h} \cdot \sum_h \left((\beta_{h,t=0} \cdot \bar{w}_{h,t=1}) - \beta_{h,t=0} \right), \quad (5)$$

$$\Delta\beta_p = \frac{1}{n_h} \cdot \sum_h \left(\beta_{h,t=1} - \beta_{h,t=0} \right), \quad (6)$$

415

416 Finally, we calculated mean change in transmission rate due to host-parasite
 417 coevolution (*i.e.*, the non-additive component of disease evolution, $\Delta\beta_{hp}$) using eq. 2.

418 To visualise how changes in host resistance, parasite infectivity and coevolution
 419 covaried, we made bivariate plots of $\Delta\beta_h$, $\Delta\beta_p$ and $\Delta\beta_{hp}$ using vectors.

420 **Quantifying ecological variation among ponds.** We calculated mean values (and
 421 also variance for temperature) for each of the 10 ecological variables over the early
 422 half of the epidemic season (over twelve sampling dates; Julian days 106-200).

423 Initially, we tested the effects of mixing treatment and then fitted separate linear
 424 models to examine the relationships between these ten variables and each of $\Delta\beta_h$, $\Delta\beta_p$
 425 and $\Delta\beta_{hp}$; we evaluated the statistical significance of these relationships after
 426 applying a sequential Holm-Bonferroni adjustment for multiple comparisons³³. Next,

427 we conducted a Principal Components Analysis (using the R function *princomp*³⁴) on
428 the ten biotic and abiotic environmental variables to generate a multivariate measure
429 of ecological variation across the pond populations (Fig. S1). We identified the first
430 four principal components as the minimum number of principal components
431 necessary for explaining over 80% of the combined variation, following standard
432 practice³⁵, and used these in subsequent analyses. For outlier detection, we calculated
433 the squared Mahalanobian distances of each population from the mean and compared
434 these values to the critical threshold for Mahalanobis' distance based on a χ^2
435 distribution, with a critical α value of 0.05. We found that all populations were below
436 the threshold value for outlier detection and thus all of populations were retained.

437 **Testing for associations between ecological variation and (co)evolutionary**
438 **trajectories.** We conducted two separate analyses to test for relationships between
439 variation in disease coevolutionary trajectories and wider ecological variation. First,
440 we tested whether pairwise differences in ecological conditions among populations
441 were associated with pairwise differences in disease coevolutionary trajectories. We
442 calculated population differences in ecological conditions (δ_{eco}), made up of the first
443 four principal components (over 80% of combined variation), using the Mahalanobian
444 distances between all of the possible pairwise comparisons of populations and the R
445 package *StatMatch* v1.3.0³⁶. We then calculated the overall multivariate distances for
446 net disease coevolution (δ_{coevo}), *i.e.*, differences in change in parasite transmission
447 rates as a composite for differences across three dimensions: host evolution, parasite
448 evolution and coevolution. We then tested for a relationship between δ_{eco} and δ_{coevo}
449 using a Mantel test fitted using the *ecodist* package³⁷.

450 Second, we used Structural equation modelling (SEM) to dissect the various
451 relationships between ecological variation, epidemic size and the components of

452 coevolution. This was done using the *piecewiseSEM* package v2.0.2 in R³⁸. SEM
453 allows the evaluation of different causal pathways between variables, and therefore
454 can evaluate support for alternative mediating variables that produce similar
455 associations. We specified two global SEMs (see Fig. S2, Table S3) with the
456 following variables; mixing, ecological variation (PC1 of the previously described
457 PCA), epidemic size, change in host resistance ($\Delta\beta_h$), change in parasite infectivity
458 ($\Delta\beta_p$) and coevolution ($\Delta\beta_{hp}$). The hypothetical causal relationships between the
459 variables included in these SEMs are outlined below:

460 *Mixing*: Mixing was an experimental treatment whereby six of the sixteen populations
461 were stirred on a weekly basis. We predicted that this would have a significant effect
462 on the ecological variables. For example, our previous work has shown that mixing
463 significantly changes *Daphnia* host population densities and affects epidemic size³².

464 *Ecology*: Ecological variation was represented by the first principal component (PC1),
465 which explained 36.0 % of the overall variation, extracted from the PCA of the
466 multiple environmental variables measured during the pond experiment. PC1 was
467 mainly associated with low mean temperature, high chlorophyll concentrations and
468 high predator density. The positive effects of temperature and negative effects of
469 predation on parasite prevalence have been well documented in *Daphnia* disease
470 systems^{13,32,39,40}. Therefore, we predicted that our measure of ecological variation
471 would be negatively associated with epidemic size and would be associated with the
472 components of transmission rate evolution (changes in host resistance, parasite
473 infectivity and coevolution).

474 *Epidemic size*: Epidemic size (integrated parasite prevalence, calculated by
475 integrating the area under the time series of empirically determined prevalence for

476 each mesocosm) could potentially be both a cause and a consequence of host
477 evolution, parasite evolution and coevolution. There is ample evidence from previous
478 studies that epidemics exert parasite-mediated selection and can cause the evolution
479 of host resistance⁴¹⁻⁴⁴, and that rapid host evolution of resistance can bring epidemics
480 to an end⁴⁵. Given the bi-directional relationship between these variables we expected
481 that there would be covariation between epidemic size and changes in host resistance,
482 parasite infectivity and coevolution, but made no prediction about the direction of
483 causality.

484 *Change in host resistance ($\Delta\beta_h$), parasite infectivity ($\Delta\beta_p$), and coevolution ($\Delta\beta_{hp}$):*

485 We developed two SEMs to test between two hypothetical relationships between
486 epidemic size, ecology and different aspects of disease evolution. Hypothesis one is
487 that ecology directly drives both epidemic size and all three components of disease
488 evolution (Fig. S2). Hypothesis two is that ecology affects epidemic size, host
489 evolution of resistance and parasite evolution of infectivity, but that decreases in host
490 resistance (*i.e.*, increased transmission rate) should negatively affect coevolution and
491 increases in parasite infectivity should positively affect coevolution. Following our
492 prediction that the wider environment has a greater impact on hosts compared to
493 parasites, we expected that there would be asymmetry in the strength of the
494 relationship between these different components of evolution with coevolution, such
495 that hosts significantly affect coevolution more than parasites.

496 After fitting the two SEMs, we tested which provided the superior fit using Bayesian
497 Information Criterion (BIC). We chose BIC over Akaike's Information Criterion
498 (AIC) and AIC corrected for small sample sizes (AICc) because BIC has been shown
499 to better predict model performance when there is unobserved heterogeneity in the
500 data⁴⁶, which seems highly likely in both our genotype frequency and ecological

501 variable data. We then conducted Fisher's C tests (Shiple's tests of directed
502 separation⁴⁷ on the best-fitting model to discover potentially relevant relationships
503 that had been excluded from the model. Finally, in order to achieve greater statistical
504 power to test the significance of each of the proposed relationships, we divided the
505 best performing global SEM into two submodels. It should be noted that the
506 parameter estimates for each of the unidirectional relationships in the submodels was
507 identical to the corresponding parameter estimates in the global model.

508

509 **Data availability:** All data is available on dryad doi:10.5061/dryad.qv9s4mwd6.

510 **Code availability:** All companion code is available on Dryad:

511 doi:10.5061/dryad.qv9s4mwd6. As we are actively researching these datasets, we

512 kindly ask that researchers contact us if they are planning to use the data for reasons

513 other than reproducing the findings of our paper.

514

515 **The following references appear in the Methods only.**

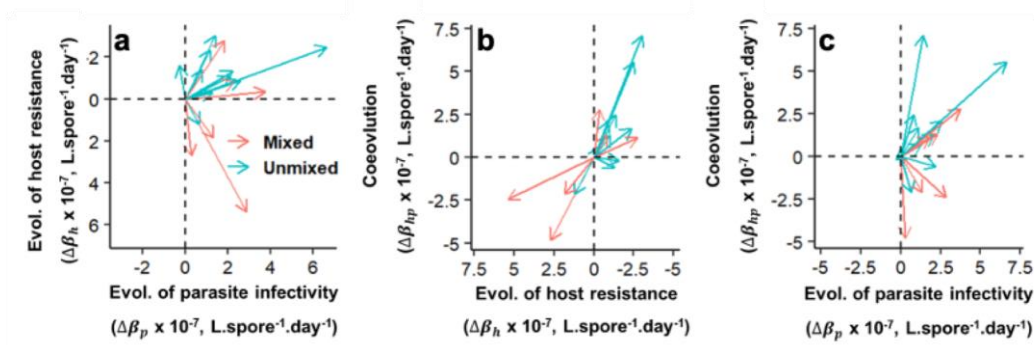
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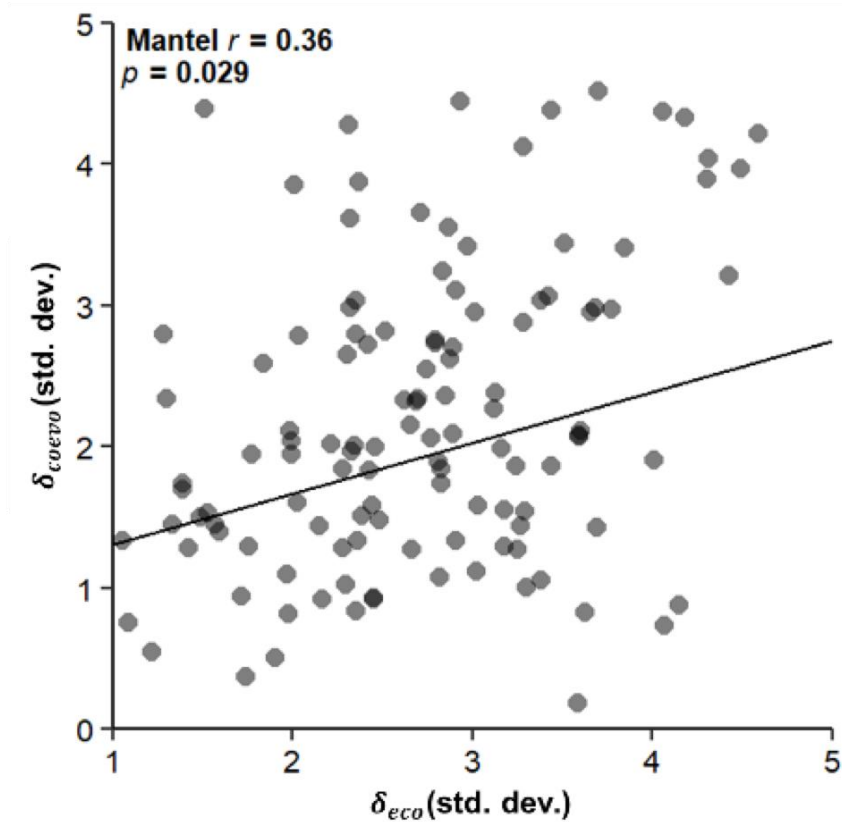


560

561 **Fig. 1. Coevolutionary trajectories vary across populations.** Vectors show pairwise
 562 relationships between **a** change in transmission rate due to host evolution of resistance
 563 ($\Delta\beta_h$) and change in transmission rate due to parasite evolution of infectivity ($\Delta\beta_p$), **b**
 564 host evolution of resistance ($\Delta\beta_h$) and non-additive change in transmission rate due to
 565 coevolution ($\Delta\beta_{hp}$) and **c** parasite evolution of infectivity ($\Delta\beta_p$) and coevolution
 566 ($\Delta\beta_{hp}$). Populations were identical pre-epidemic (vector tails) and by the end of the
 567 epidemic phenotypes had diverged due to variation in evolutionary trajectories (vector
 568 heads, open arrowheads). Red arrows denote populations that underwent the mixing
 569 treatment and blue arrows denote populations that remained unmixed.

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571



572

573 **Fig. 2. Pairwise ecological differences explain population divergence in**

574 **coevolutionary trajectory.** Relationship between pairwise population distances

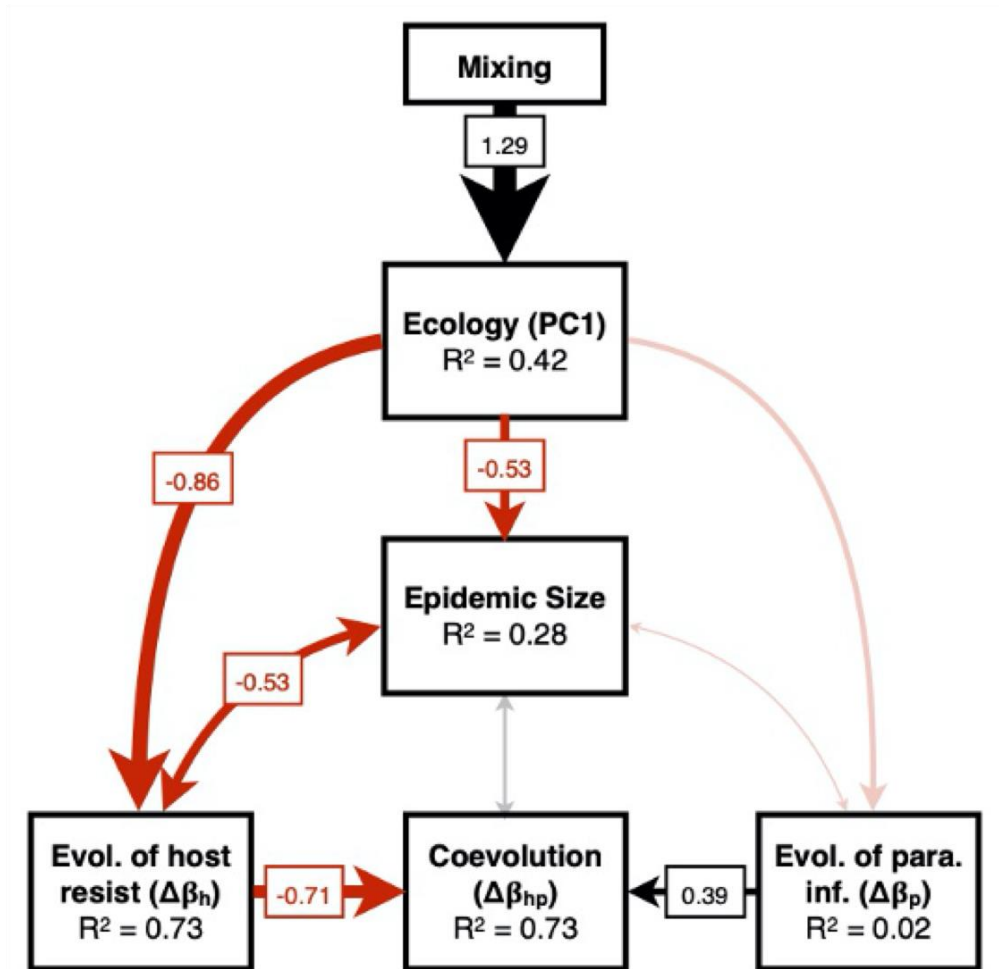
575 (measured as Mahalanobis distances) for ecology (across PC1-PC4, δ_{eco}) and net

576 coevolutionary trajectory (combining the three axes of host evolution, parasite

577 evolution, coevolution, δ_{coevo}). Pairwise differences are measured in standard

578 deviations of the total variation.

579



580

581 **Fig. 3. Wider ecology drives coevolution through its effects on host evolution.**

582 Path diagram for SEM2 showing how ecology drives coevolution. Arrows represent

583 unidirectional (single arrowhead) or bidirectional (double arrowheads) relationships.

584 Black arrows denote positive relationships, red arrows negative ones. Significant

585 ($p < 0.05$) and non-significant relationships are represented by solid and partially

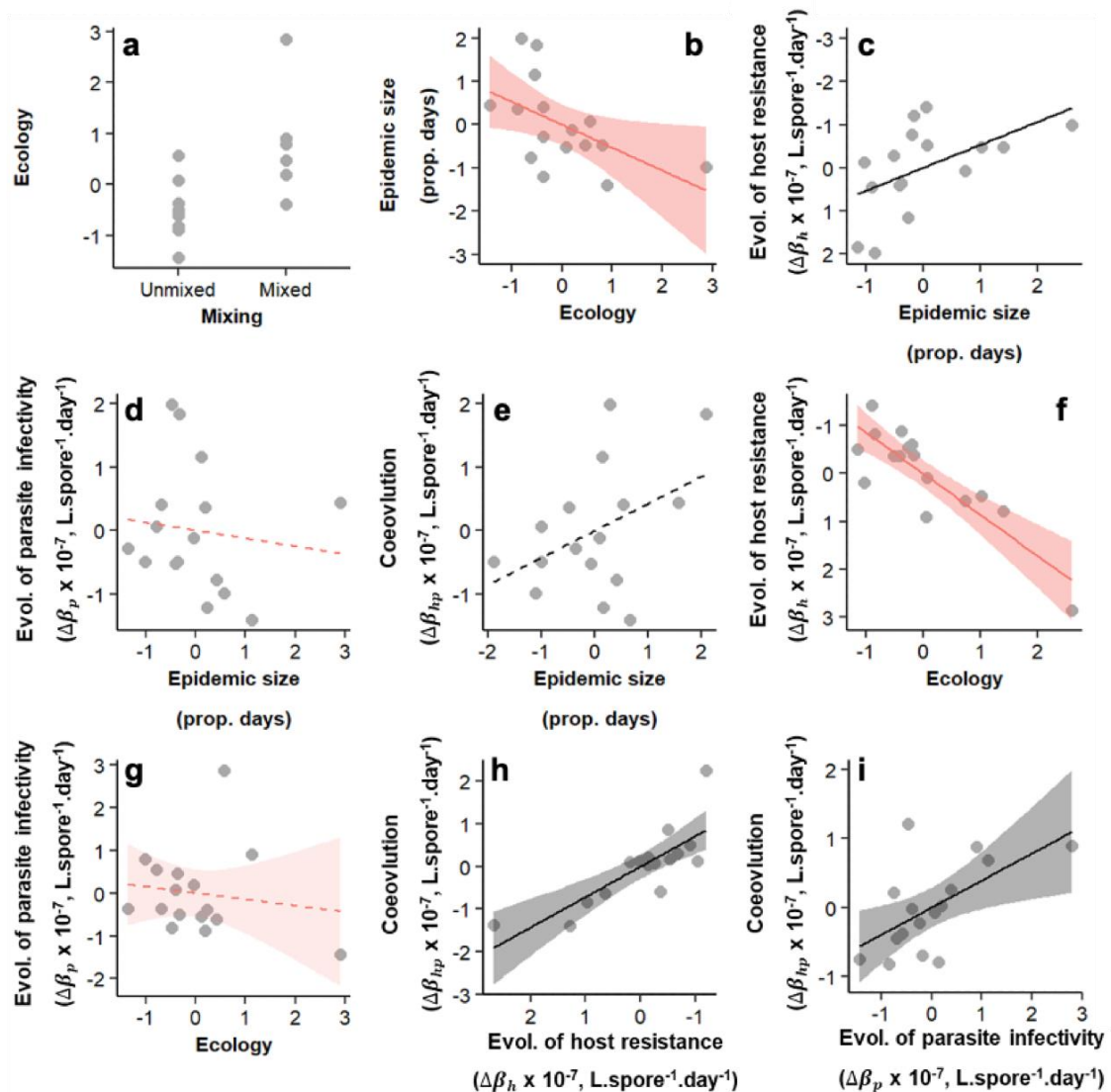
586 transparent arrows respectively. The arrow width of significant relationships is scaled

587 according to the standardised regression coefficient shown in the small boxes (see

588 also Fig. 4, Table S3). Note that negative values of $\Delta\beta_h$ represent evolution of host

589 resistance.

590



592

593

594 **Fig. 4. Ecological, epidemiological and coevolutionary relationships across**
 595 **populations.** Relationships between variables from SEM2 **a-i**. Colours show positive
 596 (black) and negative (red) relationships, and bands denote 95% CIs. Note that
 597 negative values of $\Delta\beta_h$ represent evolution of host resistance. Significant ($p > 0.05$) and
 598 non-significant relationships are indicated by solid and dashed lines respectively.

599