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Parasites driving host diversity: incidence of disease correlated with *Daphnia* clonal turnover

Short running title: Red Queen dynamics in *Daphnia*

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Author contribution: PS and JW applied for funding and designed this study together with PT. EK, BK, CT and JW contributed to sampling. EK, BK, PS, JW and CT carried out the allozyme work. EK, CT, NT and PT carried out the microsatellite work. EK, JW, CT and NT screened field samples for infection. EK measured *Daphnia* demographic parameters. PT performed all analyses. PT and JW wrote manuscript, with help of PS. All authors read and approved the final version of the manuscript and Supporting Information.

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Data archival location upon acceptance: microsatellite data will be deposited in DRYAD.

32

33 ***Abstract***

34 According to the Red Queen hypothesis, clonal diversity in asexual populations could be
35 maintained by negative frequency-dependant selection by co-evolving parasites. If common clones
36 are selected against and rare clones gain a concomitant advantage, we expect that clonal turnover
37 should be faster during parasite epidemics than between them. We tested this hypothesis exploring
38 field data of the *Daphnia* – *Caullerya* host-parasite system. The clonal make-up and turnover of the
39 *Daphnia* host population was tracked with high temporal resolution from 1998 until 2013, using
40 first allozyme and later microsatellite markers. Significant differences in the clonal composition
41 between random and infected sub-samples of *Daphnia* populations were detected on six of seven
42 tested occasions, confirming genetic specificity of the host-parasite interaction in this system. We
43 used time series analysis to compare the rates of host clonal turnover to the incidence of parasitism,
44 and found that *Caullerya* prevalence was significantly associated with microsatellite-based clonal
45 turnover. As alternate hypotheses, we further tested whether turnover was related to a variety of
46 biotic, abiotic, and host demographic parameters. Other significant correlates of turnover were
47 cyanobacterial biomass and (weakly) temperature. Overall, parasitism seems to be a strong driver of
48 host clonal turnover, in support of the Red Queen hypothesis.

49

50 ***Keywords***

51 *Caullerya*, host-parasite coevolution, Red Queen

52 ***Introduction***

53 Natural populations of asexual organisms are often very diverse (Ellstrand and Roose 1987; Duncan
54 and Little 2007; King et al. 2011), despite the variety of mechanisms of clonal loss. For example,
55 Muller (1964) predicted that clonal lineages should accumulate deleterious mutations without the
56 possibility of recombination. In general, clonal lineages are less able to respond to selection as they
57 do not produce variable progeny. One proposed mechanism for the maintenance of clonal diversity
58 is negative frequency-dependent selection by co-evolving parasites (Lively and Howard 1994). In
59 this scenario, also known as the Red Queen hypothesis (Hamilton 1980; Bell 1982), rapidly
60 evolving parasites are selected to attack the most common host clones, removing them from the
61 population and allowing other clones to rise in frequency (Lively and Morran 2014). This
62 phenomenon requires genetic specificity in host defense and parasite infectivity, meaning that the
63 outcome of infection depends on the genotypic identity of both host and parasite (Hamilton 1980;
64 Dybdahl et al. 2014). Accordingly, host genotypes should be infected disproportionally to their
65 abundance. In particular, theory predicts that common host genotypes should be either over- or
66 under-infected, depending on the phase of the oscillatory cycle, whereas the actual time of a
67 proportionate infection of common clones would be rather small (Dybdahl and Lively 1995; Kaltz
68 and Shykoff 1998). Parasites must additionally impose significant costs on infected individuals
69 (Lively and Howard 1994). The Red Queen hypothesis has a solid theoretical grounding (Hamilton
70 1980; Hamilton et al. 1990; Salathe et al. 2008; Brockhurst et al. 2014; Rabajante et al. 2016).
71 Empirical studies, however, are often challenging (reviewed in Lively and Morran 2014), because
72 of the difficulty and expense of mounting long-term high resolution field surveys.

73 The requirements for Red Queen dynamics are met in the clonally reproducing planktonic
74 crustacean waterflea *Daphnia* (belonging to *D. longispina* species complex) infected with the
75 ichthyosporean gut parasite *Caullerya mesnili* (Lohr et al. 2010a). *Caullerya* is one of the most
76 common microparasites infecting *Daphnia* of large European lakes (Wolinska et al. 2007; 2011).
77 First, experimental work has shown that *Daphnia* clones differ in their susceptibility to *Caullerya*

78 (Wolinska et al. 2006; Schoebel et al. 2010; Tellenbach et al. 2016). Moreover, in a previous three-
79 month survey of a *Daphnia* population infected with *Caullerya* in lake Rimov (Czech Republic),
80 random and infected sub-samples of *Daphnia* population significantly differed in their clonal
81 composition (Yin et al. 2012), suggesting genetic specificity of parasite infection. In a similar snap-
82 shot survey of multiple lakes (located in Switzerland and North Italy), the most common *Daphnia*
83 clone often suffered disproportionate infection, compared to other clones (Wolinska and Spaak
84 2009), also pointing towards the genetic specificity of the host-parasite interaction in this system.
85 Second, *Caullerya* is extremely virulent for its host, as it reduces survival and reproduction of
86 infected *Daphnia*, the latter by up to 95% (Wolinska et al. 2006; Lohr et al. 2010b). These
87 characteristics make the *Daphnia*-*Caullerya* system ideal for studying the effect of parasites on
88 maintenance of clonal diversity and, in particular, on clonal turnover. As *Daphnia's* generation time
89 is only one to three weeks (depending on temperature, Spaak and Hoekstra 1995) this would allow
90 observations of clonal turnover, hypothesized to be driven by parasites, within relatively short time
91 periods. It is also worth mentioning that genetic and molecular mechanisms behind infections were
92 recently discovered for another *Daphnia* species (*D. magna*) infected with a bacterial microparasite;
93 the observed patterns were consistent with host and parasite specificity assumed by the Red Queen
94 theory (Luijckx et al. 2012; Metzger et al. 2016; Bento et al. 2017).
95 Clonal diversity is a dynamic equilibrium between clonal emergence, clonal erosion, and
96 population dominance structure. *Daphnia* propagate mainly clonally, only sometime switching to
97 sexual reproduction. In temperate lakes inhabited by the *D. longispina* complex (Seda et al. 2007;
98 Keller et al. 2008; Ma et al. 2015), new clones hatch from eggs deposited in the sediment early
99 spring and reproduce parthenogenetically until winter, whereas short periods of sexual reproduction
100 occur in late spring and autumn, resulting in the release of sexual overwintering eggs (Keller and
101 Spaak 2004; Keller et al. 2007). Thus, *Daphnia* clonal emergence is directly controlled by autumn
102 (and late spring) sex and early spring hatching from resting eggs. Clonal erosion may be influenced
103 by a variety of environmental and ecological factors, including selective and neutral processes

104 (Vanoverbeke and De Meester 2010). Rare clones, particularly, are at greater risk of loss than
105 common clones, all else being equal. Similarly, the dominance structure of the population may be
106 dynamic, with different clones undergoing positive or negative selection according to shifting
107 ecological and environmental variables, resulting in rapid changes of host population structure
108 (Rabajante et al. 2016). Co-evolving parasites have the potential to affect the rate of clonal erosion
109 and to alter clonal dominance; rare clones can gain a selective advantage, perhaps lessening the rate
110 of their loss, while common clones would be selected against and removed from the population.
111 Selection against common clones was for example shown in *Daphnia*-microparasite systems
112 (Decaestecker et al. 2007; Duncan and Little 2007; Wolinska and Spaak 2009) and in freshwater
113 snails infected with trematodes (Dybdahl and Lively 1998; Jokela et al. 2009). Due to the rapid
114 generation time of *Daphnia*, it is unlikely that all parts of the co-evolutionary cycle (rare advantage,
115 increase, dominance, common disadvantage, decline) can be captured. Nevertheless, this process
116 could on average increase the diversity of the host population (Wolinska and Spaak 2009; King et
117 al. 2011; Dagan et al. 2013) and, similarly, the rate of change of the population structure – what we
118 here refer to as clonal turnover.

119 To date, studies linking parasitism with clonal turnover have mainly focused on cross-habitat
120 comparisons and have concluded that clonal turnover is faster in habitats with higher parasitism
121 (Paczesniak et al. 2014). Studies exploring parasites-induced fluctuations of host genotypes over
122 time were unfortunately restricted to a few time points only (Dybdahl and Lively 1995; Little and
123 Ebert 1999; Decaestecker et al. 2007; Jokela et al. 2009; Wolinska and Spaak 2009), and have
124 focused on habitats that are either parasitized year-round (e.g. Jokela and Lively 1995) or which
125 have unknown epidemiological histories (e.g. Wolinska and Spaak 2009). The effect of epidemic
126 parasitism on host clonal turnover has not been established, and no study of this type has amassed
127 data totalling more than a few generations. Over time, we would expect a signature of increased
128 clonal turnover during parasite epidemics Epidemics of *Caullerya* in *Daphnia* populations are
129 seasonal (lasting approximately 2 months), mostly occurring during the autumn, and often reaching

130 prevalence of 30%-40% (Wolinska et al. 2011; Tellenbach et al. 2016). This strong parasite
131 seasonality as well as its high abundance offers a unique possibility of the comparison of clonal
132 turnover between epidemic and non-epidemic months.

133 We investigated the association between host clonal turnover and parasite prevalence in a
134 14-year field study, the longest of its kind. *Daphnia* inhabiting lake Greifensee, a habitat
135 experiencing regular autumn epidemics of *Caullerya* (González-Tortuero et al. 2016; Tellenbach et
136 al. 2016), were genotyped using allozyme markers from 1998 until 2011, and microsatellite markers
137 from 2008 until 2013. First, however, we tested an important requirement of the Red Queen
138 hypothesis, namely the genetic specificity of infection. Specifically, we compared the distribution of
139 clones in random and infected sub-samples of *Daphnia* population and assessed the infection level
140 of the most common clones. Our expectation was that the genetic composition should differ
141 between these two sets of samples, due to *Caullerya* specialisation towards specific host, and
142 therefore the incidences of proportionate infections of common clones should be rare. As this
143 important requirement was met, we hypothesised that periods of high host clonal turnover will be
144 associated with *Caullerya* epidemics. Alternatively, turnover may be affected by change of any
145 environmental or ecological variable which favors some genotypes over others. We therefore further
146 compared *Daphnia* turnover to a variety of biotic and abiotic factors that are variable through time
147 and which could plausibly have genotype-specific effects. Biotic factors tested were total algal
148 biomass and cyanobacterial biomass. Both change rapidly on a seasonal schedule (Tellenbach et al.
149 2016), and may result in quick changes to the *Daphnia* community by selecting clones with better
150 feeding efficiency (Lampert 1994) and/or tolerance for cyanobacteria (Hairston et al. 2001).
151 Moreover, recent work has shown that *Daphnia* seem to be more susceptible to *Caullerya* infection
152 while being simultaneously exposed to cyanobacteria (Tellenbach et al. 2016). Other factors tested
153 were oxygen and temperature, both having a known potential to induce clonal selection (Weider and
154 Lampert 1985; Paul et al. 2012), as well as *Daphnia* demographic parameters.
155

156 ***Methods***

157 _Sampling of physico-chemical parameters and plankton_

158 Greifensee (47.37°N, 8.68°E, Switzerland) is a medium-sized, eutrophic peri-alpine lake. It has a
159 surface area of 8.5 km^2, an average depth of 18 m, and a maximum depth of 32.3 m. The lake's
160 zooplankton is dominated by members of the *Daphnia longispina* complex: *D. galeata*, *D.*
161 *longispina* and their interspecific hybrids (Keller and Spaak 2004). Physical and chemical data have
162 been recorded since the mid-20th century by the Office of Waste, Water, Energy, and Air of the
163 Canton of Zürich (AWEL, http://www.awel.zh.ch/). Depth-stratified measurements (at the surface,
164 2.5m, 5m, 7.5m, 10m, 15m, 20m, 25m, and 30m) of temperature and dissolved oxygen were taken
165 intermittently through the 1940s, and monthly since 1950. More details can be found in Bürgi et al
166 (2003). Zooplankton and phytoplankton were sampled bi-weekly during the summer, and otherwise
167 monthly, since 1961. Zooplankton were quantitatively sampled by taking six replicate hauls from 0
168 to 30 m using a 95-µm double closing net (Bürgi 1983) and preserved in 4% formaldehyde.
169 Phytoplankton was sampled in four replicate hauls from 0 to 20 m with an integrating sampler
170 according to Schröder (1969) and preserved with Lugol's solution. Zooplankton species were
171 enumerated using a dissecting microscope. Phytoplankton and microzooplankton were quantified
172 using the technique of Utermöhl (1958) on an inverted microscope.

173

174 _Sampling of Daphnia and assessment of Caullerya prevalence_

175 Additionally to the bi-weekly/monthly monitoring of zooplankton by morphology (see above), the
176 *Daphnia* community has been routinely sampled since February 1998 for genetic (allozymes and
177 microsatellites) as well as demographic analyses (for details see Keller and Spaak 2004). Adult
178 *Daphnia* females were collected for allozyme analysis (details below) on 221 occasions, with
179 sampling intervals and sample sizes varying over time. In 1998 sampling was conducted bi-weekly
180 to monthly, with frequency increasing to approximately weekly during summer. Mean sample size
181 in 1998 was 225 animals per date (SD = 208). This approximate sampling frequency was continued

182 until 2007, after which sampling was carried out every 2 to 3 months until the end of 2011. Mean
183 sample sizes were reduced to 93 +/- 37 animals between 1999 and 2002, and reduced again to 62
184 +/-21 animals from 2002 to 2011. In total, 24’107 *Daphnia* were genotyped at all four loci. On 19
185 occasions between 2007 and 2011, a total of 1241 *Daphnia* were genotyped both for allozymes and
186 for microsatellites (details below); these collections were made quarterly. In addition to this
187 comparative genotyping, samples were microsatellite-typed on 23 other occasions between 2007
188 and 2013. These collections were made approximately every month during the summer, and every 2
189 to 3 months during the rest of the year. Mean sample size was 72 animals (+/- 28). Altogether, 3484
190 animals were genotyped for microsatellite markers. Starting in 2001, the *Daphnia* selected for
191 genetic analysis were visually screened for infection by the gut parasite *Caullerya mesnili*, using a
192 dissecting microscope (for detail description of visible signs of infections see Lohr et al. 2010a), to
193 calculate the prevalence of infection. This data series has a gap of one year (June 2005 – June
194 2006). On seven sampling occasions with high *Caullerya* prevalence, additional sub-samples were
195 genotyped for microsatellites, which consisted of infected individuals only (mean sample size was
196 71 animals (+/- 18), except for a larger sample of 171 animals in 2013).
197
198 *Genotyping of Daphnia; allozymes and microsatellites*
199 *Daphnia* were genotyped at four enzyme loci: aldehyde oxidase (AO, enzyme commission number
200 [EC] 1.2.3.1), aspartate amino transferase (AAT, EC 2.6.1.1), phosphoglucose isomerase (PGI, EC
201 5.3.1.9), and phosphoglucomutase (PGM, EC 5.4.2.1), following a protocol described in Keller and
202 Spaak (2004). Two of these markers, AAT and AO, have fixed diagnostic alleles for *D. galeata* and
203 *D. longispina* (Wolf and Mort 1986; Giessler 1997). Information from all four loci was combined to
204 assign multilocus genotype labels (MLGs). Given the number of alleles we detected (4 for PGI and
205 PGM, 3 for AO, 2 for AAT), we could potentially detect 1800 MLGs, but actually only detected
206 271. We do not refer to these MLGs as clones in consideration of their low genetic resolution. Then,
207 *Daphnia* were genotyped at 8 microsatellite loci in a multiplex protocol (DaB10/14, Dp512, SwiD1,

208 SwiD10, SwiD12, SwiD14, SwiD4, and SwiD5, Brede et al. 2006), and analyzed on a 3130XL
209 sequencer (Applied Biosystems). Microsatellite peaks were identified using STRand software
210 version 2.4.59 (Toonen and Hughes 2001). Our strategy of merging microsatellite data from
211 different sequencing runs as well as an identification of MLGs are described in Appendix 1 and
212 Appendix 2, respectively.
213
214 *Genetic specificity of* Daphnia-Caullerya *interactions*
215 The clonal composition was compared between random and infected sub-samples of *Daphnia*
216 population, per given time point, using Fisher's exact test. As seven time points were tested, we
217 applied sequential Bonferroni corrections (Rice 1989) while interpreting the results. To assess
218 whether common clones were over-, under- or proportionally-infected, the number of individuals
219 representing common clone versus the number representing other clones was compared between the
220 random and infected groups (Fisher's exact test: 2 x 2 table). This test was conducted for two most
221 common clones per sampling date. In case two (but not more) clones tied for second place, both
222 were analysed then.
223
224 *Calculation of MLG turnover*
225 We calculated temporal turnover of MLGs using Bray-Curtis dissimilarities. This index ranges from
226 0 to 1, where zero represents a population consisting of the same clones in the same relative
227 frequencies at two different time points, and one represents a population having completely
228 different clonal make-up over time. To account for the variable sampling frequency, as closely
229 spaced samples are expected to be more similar than those spaced further apart, we used Webster's
230 method (Webster 1973) for discovering community discontinuities in space or time (Legendre and
231 Legendre 1998). A two-part sliding window was passed over the data series, and all samples falling
232 into each window were summed. We then rarefied the community in each window to the depth of
233 the smaller one; i.e. we randomly selected n individuals from each pooled window where n is the

234 smaller of the total samples in each window. Finally, we computed the Bray-Curtis dissimilarity
235 between rarefied windows. We tested window sizes of 90 and 120 days. Allozyme and
236 microsatellite-based time series were analyzed separately. Allozyme turnover was calculated from
237 1998 to 2011 and microsatellite turnover from 2008 to 2013.
238
239 *Time series analysis*
240 Following Tellenbach *et al.* (2016), we used time series analysis to investigate potential drivers of
241 *Daphnia* turnover, by calculating cross-correlations between turnover and a variety of biotic and
242 abiotic factors. Because the sampling dates and frequencies varied between data series, all
243 parameters (including turnover) were smoothed using a non-parametric local polynomial LOESS
244 regression and re-sampled at regularly spaced intervals. As the choice of re-sampling intervals could
245 bias the results, we calculated the cross-correlation coefficient ρ for sampling frequencies
246 approximately +/- 10 days around the mean of the actual field sampling frequencies. For the
247 allozyme data this was approximately 20 days, and for the microsatellite data this was
248 approximately 50 days. At each sampling frequency, we similarly avoided bias potentially caused
249 by choosing a particular start date, by recalculating ρ after restarting the sampling at each of the first
250 30 days of the time series. At each combination of sampling frequency and start date, we calculated
251 ρ for lags +/- 5 steps. As ρ was highest and most ecologically plausible at lags +/- 1 step
252 (corresponding to 15 to 30 days for allozymes and 40 to 60 days for microsatellites), we focus only
253 on these results. Thus, at each lag, we calculated ρ for 20 sampling frequencies × 30 start dates, and
254 then calculated the 95% confidence intervals of these estimates.
255 We attempted to correlate *Daphnia* turnover with the following parameters: (1) *Caullerya*
256 prevalence; (2) total algal biomass (g m^{-3}), including cyanobacteria, calculated by converting
257 phytoplankton densities to biovolumes according to Bürgi (1983), and then assuming that cells have
258 the specific gravity of water (Sommer 1981); (3) cyanobacterial biomass (g m^{-3}); (4) algal
259 community turnover, calculated by first separating total algal biomass into the Chlorophytes,

260 Chrysophytes, Cryptophytes, Cyanobacteria, Centric Diatoms, Pennate Diatoms, and Dinophytes.
261 The seasonal turnover was then calculated using Webster's method with a 90 day window; (5) total
262 *Daphnia* density (ind. m^{-3}); (6) *Daphnia* demographic parameters, such as growth rate r (day^{-1}),
263 birth rate b (day^{-1}), and death rate d (day^{-1}), calculated using the Edmondson egg method
264 (Edmondson 1960), using each consecutive pairs of dates; (7) integrated temperature (°C) and (8)
265 integrated oxygen (mg L^{-1}), calculated by averaging the temperature / oxygen values over the top
266 15m of the water column.

267 All data series were smoothed prior to resampling. All smoothing was performed visually to
268 avoid over fitting. We therefore used the LOESS parameter $\alpha = 0.02$ for all series except algae
269 biomass ($\alpha = 0.03$) and *Caullerya* prevalence, temperature, and oxygen ($\alpha = 0.04$). Cross-
270 correlations were calculated where the driver series overlapped completely with the turnover series.
271 Thus, the algae, abiotic and demographic series, could be used for the whole periods of allozyme
272 and microsatellite turnover, but comparisons with *Caullerya* began in 2001. The missing data in the
273 *Caullerya* series (June 2005 to June 2006) were matched by deleting the corresponding dates in the
274 allozyme turnover series before calculating cross-correlations.
275
276 ***<u>Results</u>***

277 The clonal composition differed significantly between random and infected sub-samples of
278 *Daphnia* population, in six out of seven comparisons (Fig. 1). Common clones were infected
279 disproportionally to their frequencies in eight of 14 tested combinations (three over-infections and
280 five under-infections were detected). We did not apply a correction for multiple tests (as argued in
281 Moran 2003 and Garcia 2004; see also Wolinska and Spaak 2009), but the probability of eight tests
282 being significant due to chance alone is extremely low: 1.8×10^{-7} (Moran 2003).

283 *Daphnia* microsatellite MLG turnover exhibited a clear seasonal pattern (Figure 2a), with a
284 high in mid-summer and another near the end of each year. In contrast, allozyme MLG turnover was
285 much more erratic (Figure 2b). The allozyme MLG turnover exhibited a significant upward trend

286 over time (linear model: $F_{(1, 207)} = 58.2$, $p < 10^{-3}$, $R^2 = 0.22$). We therefore performed cross-
287 correlations with both the raw allozyme MLG turnover time series, and a de-trended series. We de-
288 trended the time series by taking the residuals of the above linear model and re-scaling them to [0,
289 1] (Cowpertwait and Metcalfe 2009).

290 *Daphnia* microsatellite MLG turnover was most highly positively correlated with prevalence
291 of *Caullerya* (Figure 2c), at all three tested lags, with a maximum at lag = 0 (ρ 95% CI: 0.41 to
292 0.48, Table 1). The second most important correlate of *Daphnia* turnover was cyanobacterial
293 biomass (Figure 2d), at lags + 1 and – 1 (95% CI: 0.32 to 0.36). Finally, the cross-correlation with
294 temperature (Figure 2e) at lag = 0 was mildly important (ρ 95% CI: 0.30 to 0.33). As all of these
295 biotic and abiotic factors are likely correlated, we did not attempt to fit a model including all of
296 them simultaneously. To clarify these correlations, scatter plots of a subset of tests are given in
297 supplementary Figures S1, S2 and S3. *Daphnia* allozyme MLG turnover, in contrast, did not
298 significantly correlate with any of the tested parameters (Table S1). De-trending the data did not
299 lead to any significant correlations (data not shown).

300

301 ***Discussion***

302 Our goal was to test in a long-term study whether epidemic parasitism affected the clonal dynamics
303 of *Daphnia* in Greifensee. As previous cross-habitat work (conducted in a similar region in
304 Switzerland and North Italy) has shown that common *Daphnia* clones are often at disadvantage in
305 infected, but not in uninfected populations (Wolinska and Spaak 2009), we predicted that clonal
306 turnover will be faster during epidemics, due to parasite-driven, negative frequency-dependant
307 selection. Our results support this prediction. First, we confirmed genetic specificity of the host-
308 parasite interaction in this system. In all but one sampling occasions we detected significant
309 differences in clonal composition between random and infected sub-samples of *Daphnia*
310 populations. Further, common clones were over- or under-infected, as opposed to being infected
311 proportionately to their frequencies, in more than half of the performed comparisons. Taken

312 together we believe that these data demonstrate the genetic specificity of infection, a prerequisite
313 for the operation of Red Queen dynamics. Then, using time series analysis, we found that clonal
314 turnover of the *Daphnia* population was correlated with parasite prevalence more than any other
315 factor. As alternative explanations, we assessed the correlation between turnover and several biotic,
316 abiotic and demographic factors. Parasitism was the best correlate for the observed clonal turnover
317 of the *Daphnia* population.

318 Our observation of a correlation between the incidence of parasitism and host clonal
319 turnover is consistent with the hypothesis that parasites actually drive this turnover. These results
320 accord with the few previous studies that have compared turnover with parasitism. Investigating
321 three different New Zealand lakes, Paczesniak et al. (2014) found stronger asexual snail turnover
322 between two sampling events in shallow habitats where trematode parasites were present, compared
323 to deep habitats where parasites were absent. An earlier study of the same host-parasite system
324 (Jokela et al. 2009) similarly showed that the most common snail clones in an infected habitat
325 disappeared over seven years. Similarly, Wolinska and Spaak (2009) examined the clonal make-up
326 of 17 *Daphnia* populations at two time periods; ten of these populations were parasitized and seven
327 were not. They found that the most common clone declined significantly more often in the
328 parasitized samples. These few studies have used only two or three time points, either during a
329 single season or spaced many years apart. In contrast, in the present study, we applied microsatellite
330 markers at 42 time points over 5 years, and allozymes at more than 200 dates over almost a decade.
331 Further, in contrast to previous cross-habitat studies, we examined a system in which parasitism
332 varies epidemically through time. Thus, we have shown for the first time that parasitism can alter
333 turnover rates of hosts over time, adding to our knowledge that turnover can be higher in parasitized
334 habitats (Wolinska and Spaak 2009; Paczesniak et al. 2014).

335 This pattern was seen with clones identified with microsatellite but not with allozymes. We
336 believe that this is for two reasons. First, allozyme genotypes have much less discriminatory power.
337 That is, many different clones have the same allozyme MLG. As a consequence, changes to the

338 clonal structure of a population may not be captured by these allozyme markers. Second, two of the
339 allozyme markers used here are species specific (Wolf and Mort 1986; Giessler 1997), which is to
340 large extent not the case for microsatellites (Yin et al. 2010). As parasites are expected to evolve to
341 attack the most common clone, regardless of species identity, and as species and their hybrids
342 fluctuate according to their own set of selective forces (Brede et al. 2009; Yin et al. 2012),
343 confounding species with clones is likely to conceal the dynamics of both. Further complicating
344 matters, other biotic and abiotic factors such as food or temperature, alter both *Daphnia* clonal
345 sensitivity to *Caullerya* infection (Schoebel et al. 2010; Schoebel et al. 2011) and the species-level
346 community structure (Spaak and Hoekstra 1995; Spaak et al. 2012). Taken together, the low
347 resolution combined with the species-specificity of these allozyme markers make it unsurprising
348 that they could not reveal selection-based clonal dynamics. In fact, this allozyme sampling program
349 was not designed to investigate clonal dynamics, but rather as a long-term species-level monitoring
350 campaign (Keller and Spaak 2004).
351 Despite the erratic nature of the allozyme turnover data, the overall rate steadily increased
352 over the measurement period. This could represent an artefact of sample size or sampling frequency,
353 both of which decreased over the years. Assuming a certain similarity of the clonal population
354 between two time points, larger samples will tend to yield more accurate measurements of this
355 similarity. By decreasing the sample size, the most likely result is that rare clones will fail to be
356 detected and the dissimilarity index will be inflated. In the same way, given a stable background
357 turnover rate, longer gaps between samples will tend to yield larger estimates of dissimilarity, all
358 else being equal. However, we addressed both of these problems by first using Webster's (1973)
359 method for discovering community discontinuities, and second, by rarefying the data prior to
360 calculating the dissimilarity, to avoid inflation via different sample sizes. These adjustments should
361 have been enough to correct for both the changing sample sizes and frequencies; however, the
362 upward trend persisted even when using very wide sampling windows (data not shown). Another
363 possibility may be that the turnover rate of the allozyme alleles themselves has been increasing.

364 These alleles, unlike microsatellites, code for proteins and cannot be considered selectively neutral.
365 They may fluctuate in frequency according to some environmental or ecological factor, but we
366 cannot speculate what that might be. Finally, given that the clonal and species turnover rates are
367 confounded, the species themselves might be experiencing increased frequency fluctuation. Overall,
368 for now, we are unfortunately unable to explain the increasing allozyme turnover.

369 Aside from *Caullerya* prevalence, the best correlate of microsatellite-based clonal turnover
370 was the biomass of cyanobacteria. It is not clear whether cyanobacteria contribute to turnover *per*
371 *se*, or via association with *Caullerya*. It has been recently shown that *Caullerya* prevalence in
372 Greifensee is correlated with cyanobacterial blooms, and experimental evidence has supported this
373 facilitation (Tellenbach et al. 2016). However, if clones of *Daphnia* are differently susceptible to
374 cyanobacteria (Hietala et al. 1997; Brzeziński 2015), as has also been shown for Greifensee
375 *Daphnia* (Drugă et al. 2016), we would not necessarily expect that cyanobacterial blooms would
376 correlate with high clonal turnover. Rather, we would expect that the algal community shift to
377 cyanobacterial dominance would be met by a *Daphnia* population shift to cyanobacteria-tolerant
378 clones. We tried to capture this scenario by calculating algae community turnover, but this
379 parameter did not significantly correlate with *Daphnia* clonal turnover. Similarly, temperature was a
380 weak correlate of *Daphnia* turnover, but again it is not clear whether temperature is a real driver or
381 merely correlated with other factors. On one hand, higher temperatures decrease *Daphnia*'s
382 generation time (Spaak and Hoekstra 1995), which should presumably accelerate clonal dynamics
383 resulting from shifting selection. On the other hand, high temperature, cyanobacterial blooms, and
384 *Caullerya* epidemics often occur together (Tellenbach et al. 2016). Unfortunately, these
385 confounding variables are the nature of field studies and remain one of their main limitations.
386 Future laboratory work may help disentangle them.

387 We observed a strong correlation between *Caullerya* prevalence and *Daphnia* turnover at
388 lags -1, 0, and 1. Our turnover calculation used two-part windows, with the turnover data plotted
389 mid-way between sampling points. When sampling is frequent, these mid-points would more

390 accurately represent point-estimates of turnover, but when sampling is many months apart the
391 localization of the point is somewhat arbitrary. Data should therefore be taken as an association in
392 time, and not a strict lagging of 1 month. We don't think our results are invalidated by the variable
393 spacing of samples. All else being equal, closer samples should show less turnover, but we see
394 exactly the opposite: turnover was highest when sampling was most frequent during the summer
395 months. Moreover, the choice of window size did not alter the results: both 90 day and 120 day
396 windows showed strong correlations for the same drivers.

397 In conclusion, this study is the first to examine clonal dynamics over a long time period in a
398 system that experiences periodic parasitism. We found, in line with Red Queen theory, that periods
399 of higher infection are correlated with higher clonal turnover in the host population. Outside of
400 epidemics, where negative frequency-dependant selection is unlikely, turnover of clones is slowed
401 down. Overall, our study shows that parasites are important for maintenance of host genetic
402 diversity, and possible explains why natural populations of asexual organisms are often very
403 diverse.

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557

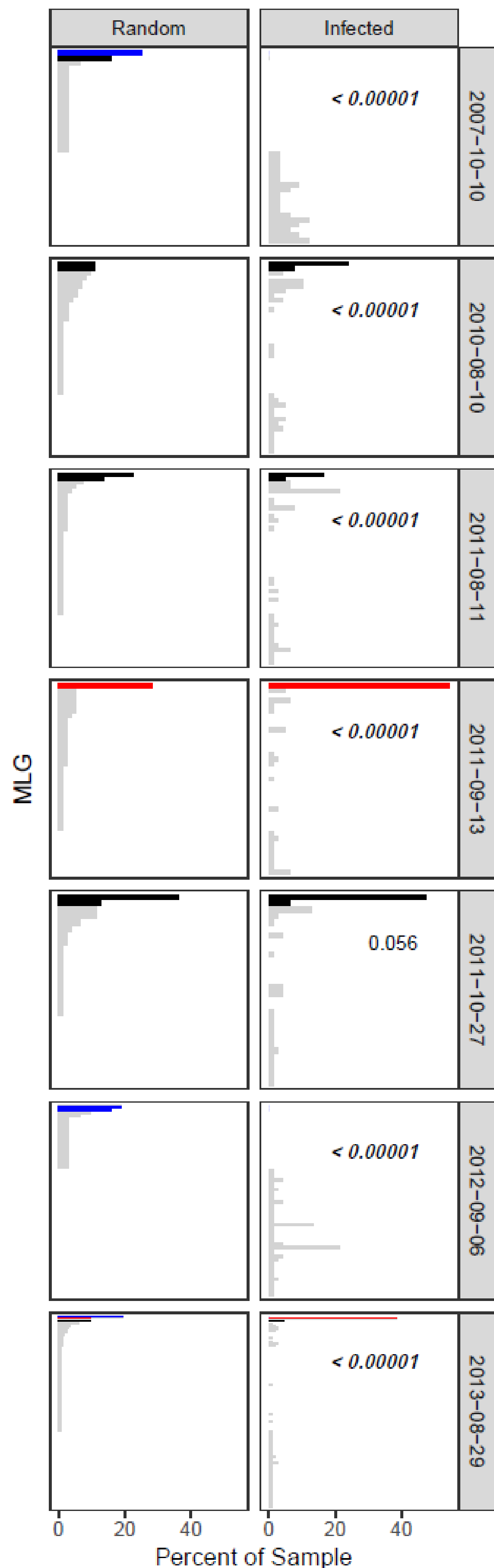
558

559 ***Tables***
560
561

Driver	Lag -1	Lag 0	Lag + 1
Total algal biomass	0.13, 0.21	0.28, 0.30	0.25, 0.30
Cyanobacterial biomass	0.32, 0.36	0.31, 0.32	0.32, 0.36
Algae community turnover	0.16, 0.23	0.27, 0.29	0.31, 0.32
Caullerya prevalence	0.37, 0.44	0.41, 0.48	0.41, 0.46
Daphnia birth rate (*b*)	0.00, 0.05	0.06, 0.10	0.08, 0.13
Daphnia death rate (*d*)	0.00, 0.09	0.11, 0.15	0.09, 0.13
Daphnia growth rate (*r*)	0.19, 0.25	0.17, 0.22	0.18, 0.24
Daphnia density	- 0.29, -0.19	- 0.04, -0.01	0.03, 0.06
Temperature	0.11, 0.21	0.30, 0.33	0.25, 0.30
Oxygen	-0.26, -0.22	-0.24, -0.22	-0.15, -0.08

562
563 **Table 1**: 95% Confidence intervals of the cross-correlation coefficient ρ between different biotic and
564 abiotic drivers of *Daphnia* microsatellite MLG turnover. Lag -1 indicates that the correlation is
565 calculated with the driver ahead of the turnover by one step of the sampling interval (40 to 60 days);
566 Lag + 1 is the opposite.
567

568 ***Figures***
569
570 **Figure 1**: Comparison of clonal composition between
571 Random (consisting of uninfected and infected
572 individuals) and *Caullerya*-infected (Infected) sub-
573 samples of the Greifensee *Daphnia* population, for 7
574 sampling dates. *P*-values (Fisher's exact tests) of
575 these comparisons are provided in the right upper
576 corners of the respective graphs. *P*-values which
577 reminded significant after applying sequential
578 Bonferroni correction are indicated in italics. In
579 addition, the two most common clones (as calculated
580 from the Random samples) were tested for over- /
581 under-representation in Infected samples.
582 Exceptionally, for the *2011-09-13* sample only one
583 common clone was tested, whereas for the *2013-08-*
584 *29* sample, three common clones were tested (see
585 main text for explanation of criteria). In red:
586 significantly over-infected clones; in blue:
587 significantly under-infected clones; in black:
588 proportionately infected clones. These data are
589 presented as percentages for ease of comparison
590 across samples, but all analyses were performed on
591 the raw counts.



592
593 **Figure 2**: Clonal turnover rates and potential drivers thereof. (a) Seasonal turnover pattern of the
594 Greifensee *Daphnia* population, as identified with microsatellites. Bray-Curtis dissimilarity was
595 calculated between groups of observations falling within 90-day windows on either side of each
596 midpoint between adjacent sample dates. (b) Increasing turnover rate of the Greifensee *Daphnia*
597 population as identified using allozymes, calculated as above. Blue line is a linear model with 95%
598 confidence interval. (c) Seasonal epidemics of *Caullerya mesnili* in Greifensee (% of *Daphnia*
599 infected). Data missing from June 2005 to June 2006. (d) Seasonal pattern of cyanobacteria abundance
600 in Greifensee (g m^{-3}). (e) Seasonal temperature fluctuation in Greifensee (°C). Temperature was
601 averaged over the top 15 meters of the lake water column.
602

603 ***Appendixes***
604

605 *Appendix 1. Merger of microsatellite data*

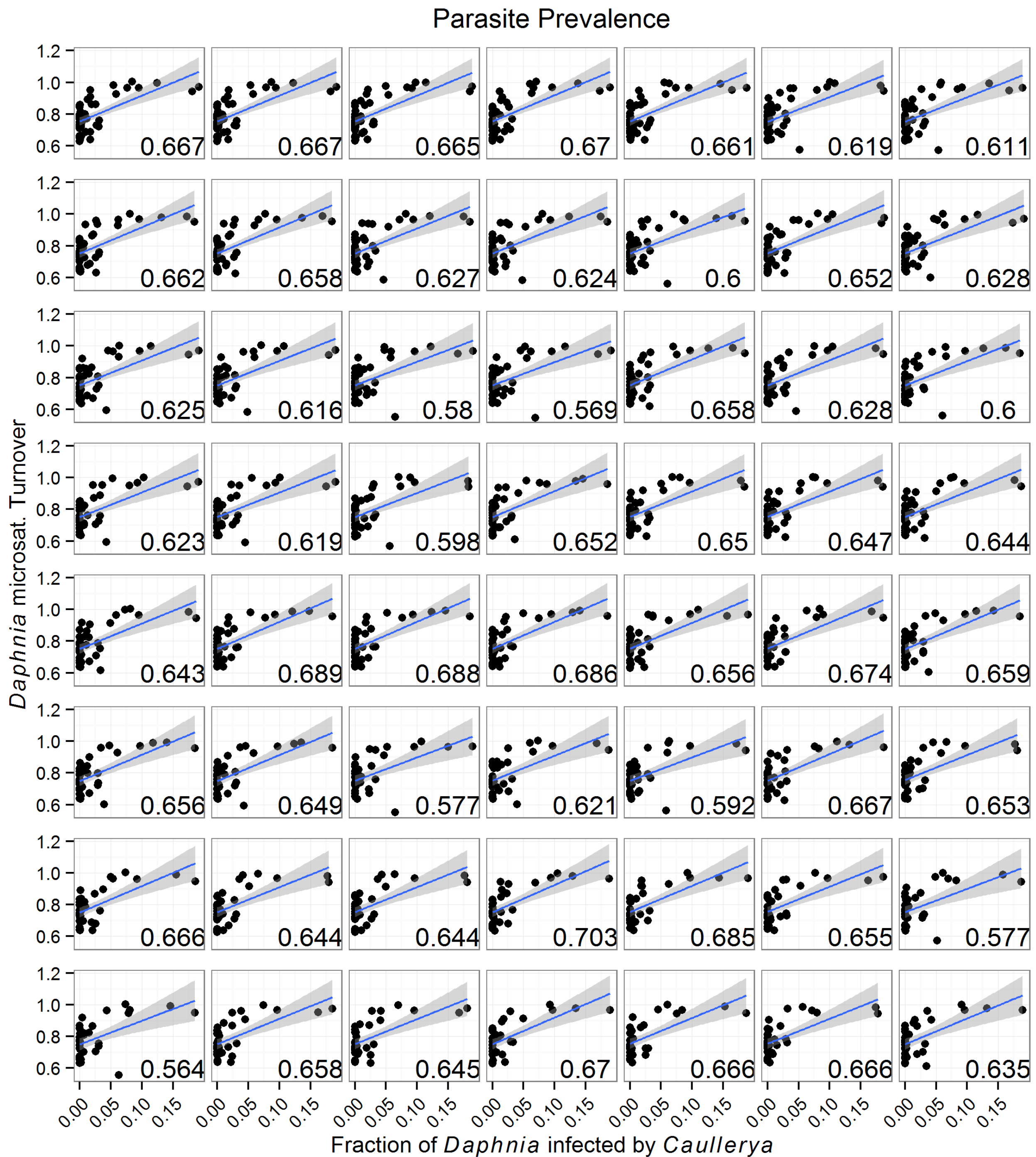
606 Microsatellite genotyping of frozen DNA was carried out between 2012 until the present. During this
607 period, several changes were made to the sequencing capillaries and the capillary polymer, resulting in
608 slight changes to the run size of the various DNA fragments. These size changes complicate the
609 assignment of integer alleles to the fragments. Several markers have alleles that differ by a single base
610 pair; slight run-length differences can therefore result in assignment of a fragment to the wrong allele.
611 We overcame this complication with a multi-stage allele binning strategy. We began by separately
612 binning fragments that were analyzed in each individual year. To do so, we used the R package
613 MSatAllele (Alberto 2009), which plots each fragment by ascending length. Discontinuous
614 distributions of these plotted lengths are taken to represent separate alleles, and the allele is labeled
615 with the integer closest to the center of each distribution. After individually binning the samples
616 genotyped in 2012, 2013, and 2014, we harmonized their allele definitions by plotting each of the
617 binning groups side by side. We could then see which years were shifted relative to which others, and
618 rename differing alleles appropriately.
619

620 *Appendix 2. Identification of multi-locus genotypes (MLGs)*

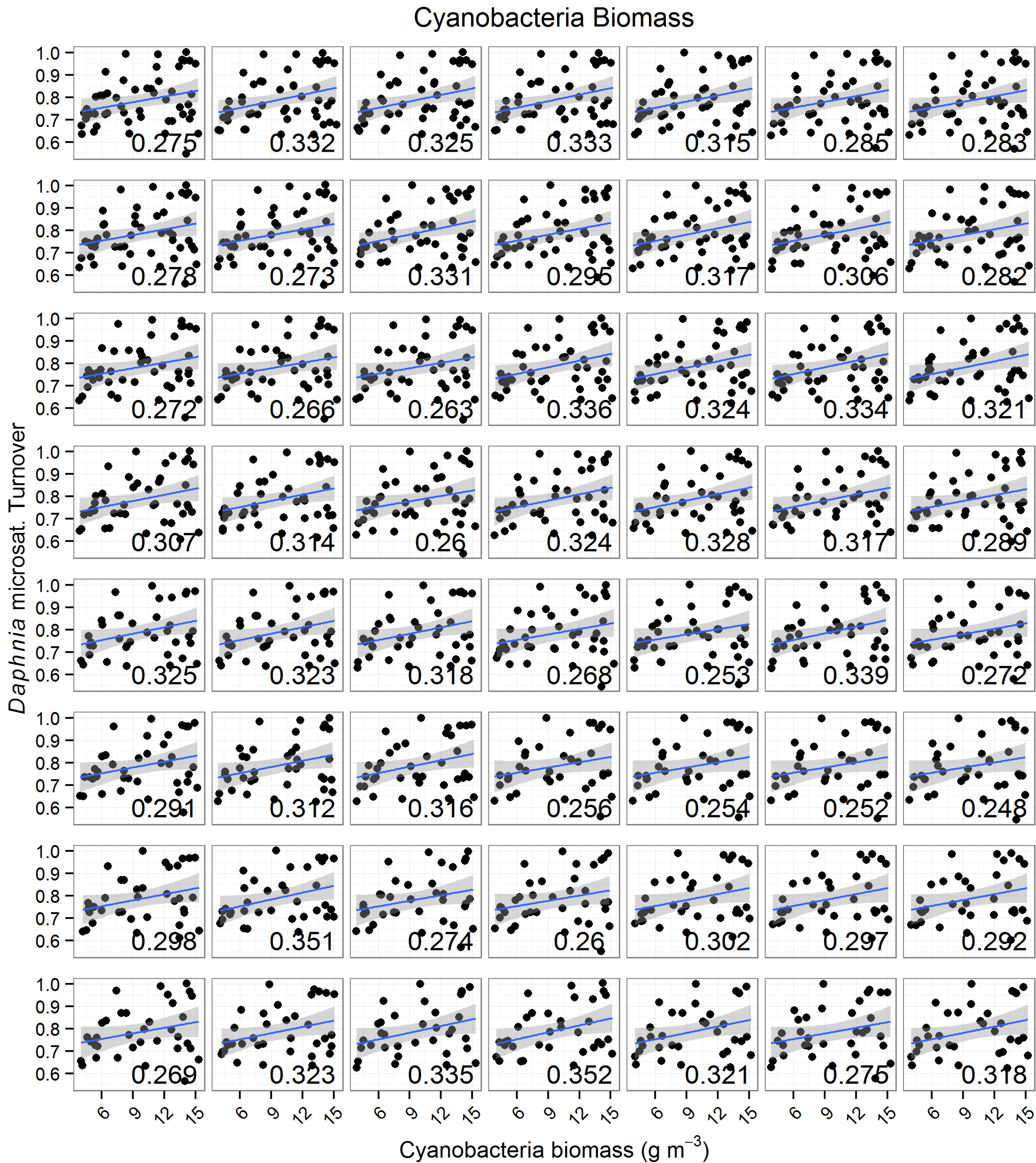
621 We first naively assigned a unique MLG label to every microsatellite genotype that differed from others
622 at a minimum of one allele at one locus, not considering missing markers. However, this procedure
623 likely overestimates the number of MLGs, given the possibility of user errors in peak identification and
624 bin assignment. User error in peak identification mainly takes the form of failing to identify one peak in
625 a heterozygous marker, resulting in spurious homozygosity. Other forms of user error, such as
626 identifying artefactual peaks as alleles or inconsistent identification of split peaks or stutter peaks, were
627 avoided by extensive training and expertise. Incorrect bin assignment could occur at the extremes of the

628 fragment length distribution of markers where alleles differ by one base pair. In this case, a fragment
629 could be wrongly assigned the label of the allele above or below it. We detected and corrected these
630 problems using partial matching. To correct for spurious homozygosity, we searched for MLGs that
631 differed from others only in that loci were homozygous instead of heterozygous (*e.g.* 200/200 instead
632 of 200/180). To detect incorrect bin assignment, we found MLGs that differed from others only in that
633 an allele differed by one base pair (*e.g.* 201/180 instead of 200/180). In both of these cases, MLGs were
634 merged under the same name if they differed only at a single locus in one of the above ways.
635 Differences at two or more loci were always taken as non-spurious. We planned to discard individuals
636 that could be partially matched to multiple MLGs, but in practice there were no such individuals. Given
637 the number of microsatellite alleles present in Greifensee, we could have discriminated $> 10^{20}$ MLGs,
638 but in fact detected only 736. Given this high resolution, we are confident that MLGs represent
639 individual clones.
640

641 ***Supplementary Material***



642 **Figure S1:** Correlation of *Daphnia* MLG turnover (microsatellites) with prevalence of *Caullerya*. As
643 both time series had to be smoothed prior to correlation, and as the specific smoothing parameters
644 could bias the results, we performed 600 smoothings within a reasonable parameter space. See text for
645 details. Shown here are a random selection of scatterplots from this 600. The cross-correlation
646 coefficient ρ at lag = 0 is given in the bottom right of each panel. The 95% CIs of all 600 ρ at lags -1, 0,
647 and 1 are reported in the results. The thin blue line is a linear regression; the grey bands are the 95%
648 CI.



649
650
651 **Figure S2:** Correlation of *Daphnia* MLG turnover (microsatellites) with cyanobacterial biomass. As
652 both time series had to be smoothed prior to correlation, and as the specific smoothing parameters
653 could bias the results, we performed 600 smoothings within a reasonable parameter space. See text for
654 details. Shown here is a random selection of scatterplots from this 600. The cross-correlation
655 coefficient ρ at lag = 0 is given in the bottom right of each panel. The 95% CIs of all 600 ρ at lags -1, 0,
656 and 1 are reported in the results. The thin blue line is a linear regression; the grey bands are the 95%
657 CI.
658

659
660

661 **Figure S3:** Correlation of *Daphnia* MLG turnover (microsatellites) with the average temperature of the
662 top 15 m of Greifensee. As both time series had to be smoothed prior to correlation, and as the specific
663 smoothing parameters could bias the results, we performed 600 smoothings within a reasonable
664 parameter space. See text for details. Shown here is a random selection of scatterplots from this 600.
665 The cross-correlation coefficient ρ at lag = 0 is given in the bottom right of each panel The 95% CIs of
666 all 600 ρ at lags -1, 0, and 1 are reported in the results. The thin blue line is a linear regression; the grey
667 bands are the 95% CI.
668

669

670

Driver	Lag -1	Lag 0	Lag + 1
Total algal biomass	-0.21, -0.15	-0.25, -0.25	-0.29, -0.28
Cyanobacterial biomass	-0.19, -0.17	-0.21, -0.21	-0.25, -0.23
Algae community turnover	0.07, 0.14	0.02, 0.02	-0.06, -0.01
Caullerya prevalence	-0.01, -0.08	-0.10, -0.09	-0.13, -0.11
Daphnia birth rate (*b*)	-0.09, -0.09	-0.11, -0.10	-0.09, -0.08
Daphnia death rate (*d*)	-0.15, -0.13	-0.18, -0.17	-0.20, -0.19
Daphnia growth rate (*r*)	-0.12, -0.10	-0.12, -0.11	-0.10, -0.09
Daphnia density	-0.05, 0.02	0.10, 0.11	0.15, 0.19
Temperature	-0.15, -0.01	-0.01, 0.04	-0.09, -0.05
Oxygen	-0-09, -0.05	-0.12, -0.12	-0.13, -0.13

671

672 **Table S1**: 95% Confidence intervals of the cross-correlation coefficient ρ between different biotic and

673 abiotic drivers of *Daphnia* allozymes MLG turnover Lag -1 indicates that the correlation is calculated

674 with the driver ahead of the turnover by one step of the sampling interval (15 to 30 days); Lag + 1 is

675 the opposite.

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