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Sex increases the probability of evolutionary rescue in the presence of a competitor

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Abstract

The explanation for the continued existence of sex, despite its many costs, remains one of the major challenges of evolutionary biology. Previous experimental studies have demonstrated that sex increases the rate of adaptation in novel environments relative to asexual reproduction. Whilst these studies have investigated the impact of sex on adaptation to stressful abiotic environments, the potential for biotic interactions to influence this advantage of sex has been largely ignored. Species rarely exist in isolation in natural conditions, so the impact of sex on adaptation to a stressful abiotic environment may be altered by the interactions between coexisting species.

To investigate the interplay of sex and competition on adaptation to deteriorating conditions, we allowed populations of the unicellular alga (*Chlamydomonas reinhardtii*) to evolve in an environment to which they were initially poorly adapted. We manipulated both their mode of reproduction and the presence of a competitor, and monitored population size and proportion of evolutionary rescue events for each mode of reproduction.

The results indicate that sex may be the beneficial strategy in the presence of the competitor. Sexual populations had highest probability of evolutionary rescue irrespective of the presence of the competitor. The overall advantage of sex was also manifested through higher level of adaptedness of survived sexual populations relative to asexual populations. Since competitive interactions are commonplace in nature, one of the explanations for the maintenance of sex by natural selection may be the increased rate of adaptation of sexual populations both in the presence and absence of competitors.

Key words: *Chlamydomonas reinhardtii*, sex, competition, evolutionary rescue

26 **1. Introduction**

27

28 The great majority of extant eukaryotic species are sexual (Bell, 1982). Despite its
29 ubiquity, sex still remains an unresolved challenge for evolutionary biologists to explain. This
30 is why sex has been a subject of numerous theoretical and experimental studies for more
31 than a century (Kondrashov, 1983; Burt, 2000; Hartfield and Keightley, 2012).

32 The paradox of sex is reflected in the fact that it entails various costs that are
33 disadvantageous for an individual and a population. For example, sex may result in a decline
34 of mean fitness of a population as a result of a break-up of favorable combinations of alleles,
35 termed recombination load (Barton and Charlesworth, 1998), as experimentally
36 demonstrated (for example: Greig et al., 1998; Colegrave et al., 2002; Kaltz and Bell, 2002;
37 Becks and Agrawal, 2012). In addition to such genetic costs, sex can impose direct costs as
38 well, such as investment of resources into finding a mate or increased likelihood of
39 transmission of pathogens and parasites between individuals or their gametes. Moreover, in
40 anisogamous species, sex entails the two-fold reduction of reproductive output per female as
41 a cost of producing males, which has been termed “the two-fold cost of sex” or “the cost of
42 males” (Maynard Smith, 1971; Williams, 1975; Maynard Smith, 1978). Considering all these
43 costs, the continued existence of sex implies that there must be a compensatory selective
44 advantage.

45 Most hypotheses that explain the benefits of sex gravitate towards the idea that sex
46 generates variation within a population, which is then available for selection to act on, and
47 thus enhances adaptation to a changing environment (Weismann, 1889; Fisher, 1930;
48 Muller, 1932; Felsenstein, 1974; Burt, 2000). The major advantage of sex based on this
49 concept is faster accumulation of multiple beneficial mutations within a single individual,
50 which in turn increases the response of natural selection and the rate of adaptation. In
51 contrast, the fixation of beneficial mutations would proceed at a slower rate (one at a time) in
52 asexual populations, which would ultimately result in the competition of genotypes carrying

53 different mutations (Muller, 1932), consequently slowing down adaptation (a process termed
54 clonal interference; Gerrish and Lenski, 1998).

55 Recent experimental work has shown that sex can have important consequences for
56 the rate at which a population adapts to novel environments. For instance, Kaltz and Bell
57 (2002) subjected experimental populations of *Chlamydomonas reinhardtii* to heterotrophic
58 growth on novel carbon sources and compared the rate of adaptation of sexual and asexual
59 populations. Repeated episodes of sex provided a long-term advantage in mean fitness
60 relative to asexual populations. This relative advantage of sexual populations is directly
61 proportional to population size prior to induction of sex, as demonstrated by Colegrave
62 (2002). This result suggests that sex reduces the constraint of adaptation imposed by clonal
63 interference in larger populations. Becks and Agrawal (2012) subjected sexual and asexual
64 populations of a monogont rotifer *Brachionus calyciflorus* to novel environments (elevated
65 concentration of NaCl and novel food source) and found that during the initial stages of
66 adaptation sexually derived offspring had higher fitness (measured as lifetime reproduction
67 per female) than the asexually produced genotypes. In addition to more efficient adaptation,
68 sex may decrease the likelihood of extinction. Bell (2013) found that the populations of *C.*
69 *reinhardtii* with a history of obligate sexual reproduction have higher probability of survival
70 relative to asexual populations, when subjected to growth in the absence of light.

71 A growing body of experimental studies demonstrates that sex is also beneficial in
72 deteriorating environments. Goddard et al. (2005) compared the rate of adaptation between
73 sexual and asexual yeast populations in benign and deteriorating conditions (an increase of
74 osmolarity and temperature). Relative fitness of sexual populations (measured as growth
75 rate) was significantly higher in the deteriorating environment, but not in benign conditions.
76 Sex may also increase the likelihood for survival of a population in conditions that would
77 have ultimately caused extinction of an ancestral population, a process termed “evolutionary
78 rescue” (Gomulkiewicz and Holt, 1995). Lachapelle and Bell (2012) manipulated genetic
79 diversity and mode of reproduction (obligate sexual, facultative sexual and asexual) of
80 *Chlamydomonas reinhardtii* populations, and monitored the extinction dynamic while the

81 environment gradually deteriorated (an increase of salinity). They found that a combination
82 of obligate sexuality and high genetic diversity significantly increased the survival rate.
83 Furthermore, high-diversity sexual populations had significantly higher level of adaptation to
84 high salt concentrations relative to other treatment groups.

85 Previous experimental studies have focused on the impact of sex on adaptation to a
86 stressful abiotic environment. However, these studies have largely ignored the potential for
87 biotic interactions to influence the advantage of sex. Investigating the effect of sex within a
88 multispecies context is essential, because in natural conditions species rarely exist in
89 isolation. Hence, the ability to adapt and the effect of sex on adaptation may depend on
90 interactions between coexisting species.

91 The effects of sex in the presence of other species have been mostly studied within a
92 parasite-host system. Lively (1992) found positive correlation between the frequency of
93 males in a facultative sexual species *Potamopyrgus antipodarum* and the presence of
94 parasites (trematodes). Morran et al. (2009) provided experimental evidence for increased
95 level of outcrossing in the wild-type population of *Caenorhabditis elegans*, when subjected to
96 a bacterial pathogen (*Serratia marcescens*). However, while these studies have been
97 focused on the biotic factors which increase the frequency of sex, to our knowledge, no
98 experimental study has investigated whether the effects of sex on adaptation are altered by
99 the presence of other species.

100 Here, we focus on how competitive interactions may modulate any effects of sex on
101 adaptation. We focus on the competitive (negative) interactions between species, because
102 recent studies (Johansson 2008; Collins 2011) demonstrated that competition restricts
103 adaptation and so might be predicted to constrain the beneficial effects of sex. Johansson
104 (2008) modeled competition between two species in a changing environment and found that
105 the species disfavored in competition suffers a decline in effective population size, which in
106 turn reduces the maximal rate of adaptation. This result was corroborated by experimental
107 study of Collins (2011). She demonstrated that the rate of adaptation (absolute fitness) of
108 *Chlamydomonas reinhardtii* to elevated concentration of CO₂ declines in presence of a

109 competitor, due to a trade-off between adaptation to abiotic and biotic component of
110 environment.

111 Interplay of sex and competition may result in several outcomes. Firstly, the
112 beneficial effect of sex may increase with an increase of complexity of environment
113 (incorporation of a competitor into the environment of a focal species). Adaptation to
114 complex environments may involve multiple loci (Kaltz and Bell, 2002). Under these
115 conditions, sex may allow for more efficient assembling of multilocus genotypes, thus
116 increasing the fitness advantage of sexual over asexual populations (Kaltz and Bell, 2002).
117 However, the presence of a competitor is likely to cause a decline of population size of
118 adapting population, thus reducing the supply of beneficial mutations. Consequently, the rate
119 of adaptation may decline irrespective of mode of reproduction of a population. Under these
120 conditions, sex may still remain the beneficial strategy, but relative advantage of sexual
121 populations (an increased rate of adaptation) diminishes. Alternatively, sex may be
122 disfavored if negative effects of competition are augmented by the genetic costs such as
123 recombination load. A decline in population size caused by competition, coupled with a
124 short-term decline of mean fitness of a population may outweigh the benefits of sex and thus
125 favor asexual mode of reproduction.

126 In order to investigate the combined effect of sex and competition on adaptation to
127 changing (deteriorating) conditions, we allowed populations of the unicellular green alga
128 (*Chlamydomonas reinhardtii*) to evolve in an environment to which they were initially poorly
129 adapted. Under these conditions, the populations decline and, in the absence of evolutionary
130 change, the populations will go extinct. We manipulated both their mode of reproduction
131 (either entirely asexual, or with a history of sexual reproduction) and the presence of a
132 competitor. We monitored population size and proportion of surviving populations (the
133 number of evolutionary rescue events) for each mode of reproduction throughout the course
134 of experiment.

135 If sex is beneficial in both the presence and absence of a competitor, we predicted a
136 higher number of evolutionary rescue events in sexual populations, irrespective of the
137 competition treatment. If the advantage of sex is more pronounced in the presence of the
138 competitor, the relative proportion of evolutionary rescue events of sexual populations will
139 increase in the presence of the competitor. Finally, if sex is disadvantageous in the presence
140 of the competitor, we predicted a lower proportion of evolutionary rescue events relative to
141 asexual populations.

142

143 **2. Materials and methods**

144

145 ***Base Populations of Chlamydomonas reinhardtii***

146 In order to establish genetically variable experimental populations of the focal
147 species, *C. reinhardtii*, the mass mating of 10 different wild types strains was performed. The
148 zygotes obtained from the mass mating were plated on agar and allowed to germinate by
149 producing zoospores. The zoospores differentiated into adult vegetative cells which were
150 allowed to undergo several rounds of mitotic divisions until visible colonies appeared on agar
151 plates. Each colony represents a single (unique) genotype derived from an individual cell. *C.*
152 *reinhardtii* is an isogamous organism with two mating types (mt): mt+ or mt- and no mating
153 type switching. A library of 40 genotypes was established by randomly picking 20 colonies of
154 each mating type from the agar plates by sterile loops. The mating type was determined by
155 crossing the culture derived from each genotype with tester isolates. Each experimental
156 population represents a unique combination of 10 genotypes, randomly chosen from the
157 library: 48 sexual populations were established by combining 5 mt+ and 5 mt- isolates; two
158 sets of 24 asexual populations were established by combining 10 mt+ and 10 mt- isolates,
159 respectively.

160 ***The Competitor Species (Chlamydomonas moewusii)***

161 The competitor species used in this study was another unicellular chlorophyte alga,
162 *Chlamydomonas moewusii*. This freshwater species was selected for the experiment due to
163 diversity of strains isolated from natural habitats and similar growth requirements as *C.*
164 *reinhardtii*. We obtained 4 wild type strains (cc-1419, cc-1420, cc-1480 and cc-1481) from
165 the University of Minnesota algae collection (<http://www.chlamycollection.org/strains/>). All
166 wild types of the competitor species were cultivated separately throughout the course of
167 experiment, hence no rounds of sexual reproduction have been allowed.

168

169 **EXPERIMENTAL EVOLUTION**

170

171 Experimental evolution was carried out in two phases. In the first phase, populations
172 of the focal species experienced a deteriorating environment in which NaCl (hereafter
173 referred to as salt) concentration increased to levels that were expected to be lethal. In the
174 second phase, their ability to adapt to these conditions was monitored in both the presence
175 and absence of the competitor species.

176

177 ***Cultivation and Transfer Procedure***

178 All experimental populations of *C. reinhardtii* were cultivated in 24-well plates under
179 standard conditions (26°C, 3200 lux illumination, shaking at 180 rpm and covered with sterile
180 breathable membranes to prevent cross-contamination and uneven evaporation across the
181 plates). A serial passage was performed after every 3-4 days (a period hereafter referred to
182 as growth cycle, corresponding to approximately 9-12 doublings of cells per culture) by
183 transferring 5 % of each population to a fresh medium. After every second growth cycle,

184 each population was sampled (150 µl) and population size estimated spectrophotometrically
185 by measuring optical density (OD₇₅₀) of the culture. The experimental populations were
186 propagated in Bold's broth medium (2 ml per culture) supplemented with salt, which
187 concentration gradually reached 15 g/l in three equal steps of increase (5 g/l). The salt
188 concentration of 15 g/l was chosen as an endpoint because it completely inhibits the growth
189 of *C. reinhardtii* (Reynoso and de Gamboa, 1982). The stepwise increase in salt level was
190 selected as an experimental procedure since the immediate exposure to the lethal stress
191 would have likely resulted in a too abrupt decline of population density. The likelihood of
192 contact between two opposite gametes decreases in a sparse population which can
193 consequently hamper the completion of the sexual cycle (production of zygotes).

194

195 ***Sexual Cycle***

196 Prior to each step of salt increase (after every two growth cycles), sexual populations
197 were allowed to undergo a sexual cycle. We allowed three sexual cycles in total, since Kaltz
198 and Bell (2002) had demonstrated that three successive episodes of sex provide a long-term
199 increase of the rate of adaptation of sexual populations. Each sexual cycle was induced
200 using the following protocol. All the populations were centrifuged at 5000 rpm for 10 minutes,
201 re-suspended in nitrogen-free medium to initiate gametogenesis and incubated in standard
202 conditions under bright light for another 24 hours to allow for mating and formation of
203 zygotes. After given period, the plates containing experimental populations were wrapped in
204 aluminium foil and incubated in dark for additional 4-5 days to allow the zygotes to mature.
205 Since sublethal stress may affect the mutation rate, the effects of sex may be confounded
206 with the effects of mutations (Goho and Bell, 2000; Colegrave et al, 2002). Hence, the
207 nitrogen starvation treatment was also applied to asexual populations, but since they were
208 made up of single mating types, no mating took place. After incubation in darkness, the
209 plates with mature zygotes were placed in a freezer for 4 hours (-20°C) in order to eliminate

210 unmated gametes. The zygotes develop the additional layers of cell wall during maturation in
211 the darkness, thereby acquiring resistance to stressful conditions (Harris, 2009). The pilot
212 experiments in our laboratory revealed that this feature enables the zygotes to withstand low
213 temperature. Given the lack of resistance of unmated gametes to freezing, this procedure
214 was omitted for the asexual populations. The zygotes of the sexual *C. reinhardtii* populations
215 were then transferred to agar plates by sterile loops and incubated in bright light for two days
216 to allow for germination and several rounds of mitotic divisions. The asexual *C. reinhardtii*
217 populations were transferred to agar plates by pipetting (an aliquot of 150 μ l) and incubated
218 for the same interval of time. After given period, all the cultures in agar plates were flooded
219 with 4 ml of Bold's broth medium (supplemented with salt which concentration increased for
220 5 g/l than prior to induction of sexual cycle) for approximately an hour. Population size of
221 each experimental population on agar was estimated spectrophotometrically (OD_{750}) and
222 diluted to the same optical density as prior to induction of sexual cycle. This experimental
223 procedure ensured that effects of sex were not confounded with a variation in population
224 size. All the populations were then returned to the liquid medium.

225

226 ***Assay of high salt tolerance of both species***

227 The probability of evolutionary rescue of the focal species might be affected by the
228 potential differences in high salt sensitivity between *C. reinhardtii* and *C. moewusii* since the
229 species with a faster growth rate in the high salt environment (and therefore, more tolerant to
230 the stressful conditions imposed on both competitors) could reduce population size of the
231 species with a slower growth rate (relative to the treatment without the competitor). Since the
232 probability of evolutionary rescue is positively correlated with population size (Bell and
233 Gonzales, 2009), the populations of the less tolerant species may adapt less efficiently and
234 ultimately go extinct. To investigate this possibility, all 40 ancestral isolates of experimental
235 *C. reinhardtii* populations and all four *C. moewusii* wild type isolates were assayed for growth

236 in the high salt medium (Bold's supplemented with 15 g/l of salt) prior to commencing of the
237 experiment. The populations of both species were allowed two growth cycles in 96-well
238 plates (3-4 days; 5% of culture passaged), but were assayed only during the second growth
239 cycle, to avoid the possible carry-over effects after plating from agar slant tubes, used for
240 storing the cultures. The growth rate (average yield per time point) was estimated
241 spectrophotometrically, by measuring optical density (OD₇₅₀) twice a day (approximately
242 every 12 hours), until the cultures reached the stationary phase (after approximately 4 days).

243

244 ***Establishing and Cultivation of Mixed Populations***

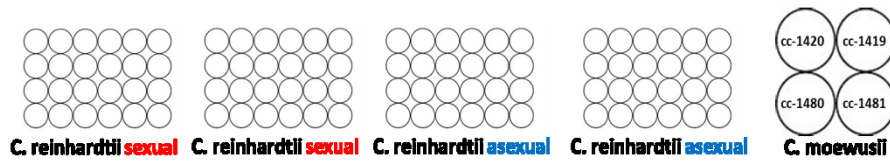
245 To examine the interplay between mode of reproduction and competition, during the
246 second phase of the experiment we manipulated the presence or absence of the competitor
247 (summarized in Figure 1, see below). The decision not to add the competitor to the first
248 phase of experiment was taken, because the freezing procedure applied during the sexual
249 cycle of *C. reinhardtii* would have been lethal for *C. moewusii*. In the second phase of the
250 experiment, at the point which the environment had reached its maximum salt concentration,
251 the mixed populations were assembled by combining each wild type of *C. moewusii* with six
252 sexual populations and six asexual populations of *C. reinhardtii*. The isolated asexual and
253 sexual *C. reinhardtii* populations (twenty-four per each mode of reproduction) were not
254 combined with the competitor. Prior to assembling with *C. reinhardtii* populations, the
255 populations of all four *C. moewusii* wild types underwent the same cultivation and transfer
256 procedure as described (see "Cultivation and Transfer Procedure" section), including the
257 three rounds of salt increase.

258 Both species of the mixed populations started this phase of the experiment with
259 equal cell densities, estimated the following way. Nine randomly chosen populations of *C.*
260 *reinhardtii* and the population of each wild type of *C. moewusii* were sampled (150 µl) and
261 population size estimated by cell counting with a haemocytometer. Each of the *C. moewusii*

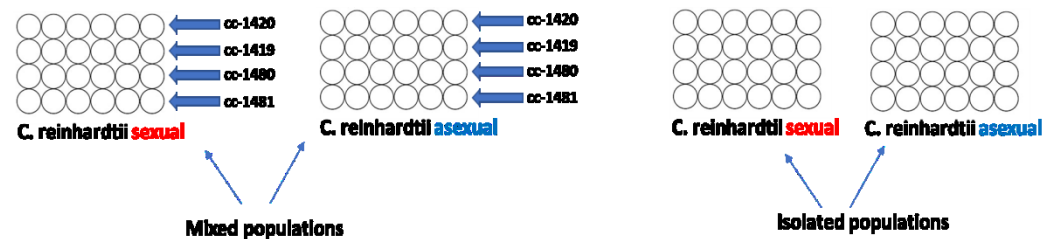
262 wild type populations were then diluted to the culture density corresponding to the average
 263 cell number of the sampled *C. reinhardtii* populations.

264

A) First phase of experiment



B) Second phase of experiment



265

266 **Figure 1 – Experimental design.** In the first phase of experiment, which lasted until salt concentration
 267 reached 15 g/l, both species were cultivated separately: two groups of sexual populations of *C.*
 268 *reinhardtii*, two groups of asexual populations of *C. reinhardtii* and a single population of each wild type
 269 of *C. moewusii*; each circle represents a single population. During this phase, populations in sexual
 270 groups of *C. reinhardtii* underwent three sexual cycles. In the second phase, each wild type of *C.*
 271 *moewusii* was combined with six populations of one sexual *C. reinhardtii* group and six populations of
 272 asexual *C. reinhardtii* group. The remaining sexual and asexual groups of populations were propagated
 273 in isolation, without the competitor.

274

275

276 All the mixed populations and control *C. reinhardtii* populations were cultivated in the
 277 Bold's broth medium supplemented with 15 g/l of salt throughout the rest of experiment,
 278 maintained by serial passage (5 % of each population) performed after every 3-4 days. Prior
 279 to each passage to the fresh medium, a sample (200 µl) of each population was serially
 280 diluted (10^{-1} or 10^{-2} - fold dilution) and transferred to the corresponding Petri dish with Bold's
 281 agar medium supplemented with sodium-acetate. Each sampled population was incubated
 282 until colonies appeared (approximately 4 days) and the cell number was estimated by colony

283 counting. The cell number of the population sample was converted to the cell number (per
284 ml) of the whole population by using the equation: cell number per ml = number of colonies /
285 dilution factor X sample size (0.1 ml). After given interval of time, all the mixed populations
286 were wrapped in aluminium foil and incubated in dark for additional 3-4 days to determine
287 the population size of each species.

288

289 ***Distinguishing the two species***

290 To distinguish the two species, we grew them under conditions in which they would
291 show different growth characteristics on agar plates. We made use of the fact that *C.*
292 *reinhardtii* grows well in the dark on acetate supplemented agar plates, whilst *C. moewusii*
293 does not.

294 Both species are facultative heterotrophs, capable of utilizing sodium-acetate while
295 incubated in light. However, *C. moewusii* cannot metabolize sodium acetate in the dark or
296 otherwise shows poor growth (Harris, 2009). In contrast, *C. reinhardtii* cells utilize sodium
297 acetate and continue dividing in the dark, although with a reduced growth rate than under
298 light conditions. This biological feature had been exploited in this experiment, given that *C.*
299 *reinhardtii* colonies clearly increased in size after incubation in darkness, thus becoming
300 distinguishable in a mixed culture. After the identification based on colony sizes, colonies
301 were additionally inspected (under inverted microscope with 20 times magnification) to
302 distinguish the two species based on the individual cell features. The cells of *C. reinhardtii*
303 are oval or spherical, while the *C. moewusii* colonies comprise the elongated and ellipsoid
304 cells (personal observation).

305

306

307

308 ***Recording the evolutionary rescue events***

309 Experimental evolution was continued until all *C. reinhardtii* populations either went
310 extinct (both in isolation and as a part of mixed populations) or showed clear positive growth
311 between transfers. The *C. reinhardtii* populations were scored as ‘rescued’ if they repeatedly
312 scored the same or higher number of cells / ml each time sampled (minimum three
313 consecutive growth cycles), thus showing positive growth in lethal conditions. The
314 populations were scored as ‘extinct’ if no cells were detected after both visual inspection of
315 liquid culture (under inverted microscope with 20 times magnification) and incubation of a
316 culture sample (200 µl, approximately 10% of a culture) plated on a Petri dish with agar.

317

318 **3. Data analysis**

319

320 The probability of evolutionary rescue was estimated by fitting a Generalised Linear
321 Model (binomial logistic regression) with two factors as categorical independent variables
322 (‘mode of reproduction’ and ‘competition’), and survival/extinction as a binary response
323 variable. The factors were treated as fixed effects. ‘Mode of reproduction’ comprises two
324 levels (sexual or asexual populations), whilst ‘competition treatment’ comprises five levels:
325 absence of the competitor and each of the four wild types being considered as a different
326 level. Since our primary question is about the interaction between mode of reproduction and
327 competition, this two-way interaction was also included in the model. Since a binomial
328 logistic regression is fitted on a log-odds scale, this interaction tests whether the relative
329 effect of sex on the odds of rescue depends on whether competition is present or absent.
330 Based on a significant overall effect of the competition treatment, this effect was further
331 explored by model simplification (Crawley, 2013). Since our main objective was to
332 investigate the differences between the absence and presence of the competitor, the full
333 model was simplified by stepwise pooling the levels within ‘competition treatment’ factor

334 corresponding to the individual wild types (starting by pooling the two competitors that have
335 caused most extinctions) until the point which further simplification was not statistically
336 significant.

337 The adaptedness of survived populations was analyzed by fitting Two-Way ANOVA,
338 with two factors as categorical independent variables ('mode of reproduction' and
339 'competition') and 'mean population size per growth cycle' as a continuous response
340 variable. The populations which survived in the presence of the competitor (irrespective of
341 the wild type) were pooled into a single level, since no evolutionary rescue events were
342 recorded in some treatment groups. Hence, 'competition' comprises two levels: presence
343 and absence of the competitor. The data was rank transformed prior to the analysis.

344 The difference in the probability of survival between *C. reinhardtii* and *C. moewusii*,
345 while being propagated in mixed populations, was tested by fitting a Generalised Linear
346 Model (binomial logistic regression) with 'species' as categorical independent variable. The
347 difference in the probability of survival among different *C. moewusii* wild types was tested by
348 fitting a Generalised Linear Model (binomial logistic regression) with 'wild type' and 'mode of
349 reproduction (of *C. reinhardtii*)' as categorical independent variables.

350 The difference in high salt sensitivity between *C. reinhardtii* ancestral populations and
351 *C. moewusii* was tested by fitting One-Way ANOVA, with 'species' as categorical
352 independent variable. The dependent continuous variable was the slope of the curve
353 representing mean change of a population size (yield) as a function of time (h).

354 All the analyses were performed using R (R core team, 2017).

355

356

357

358 4. Results

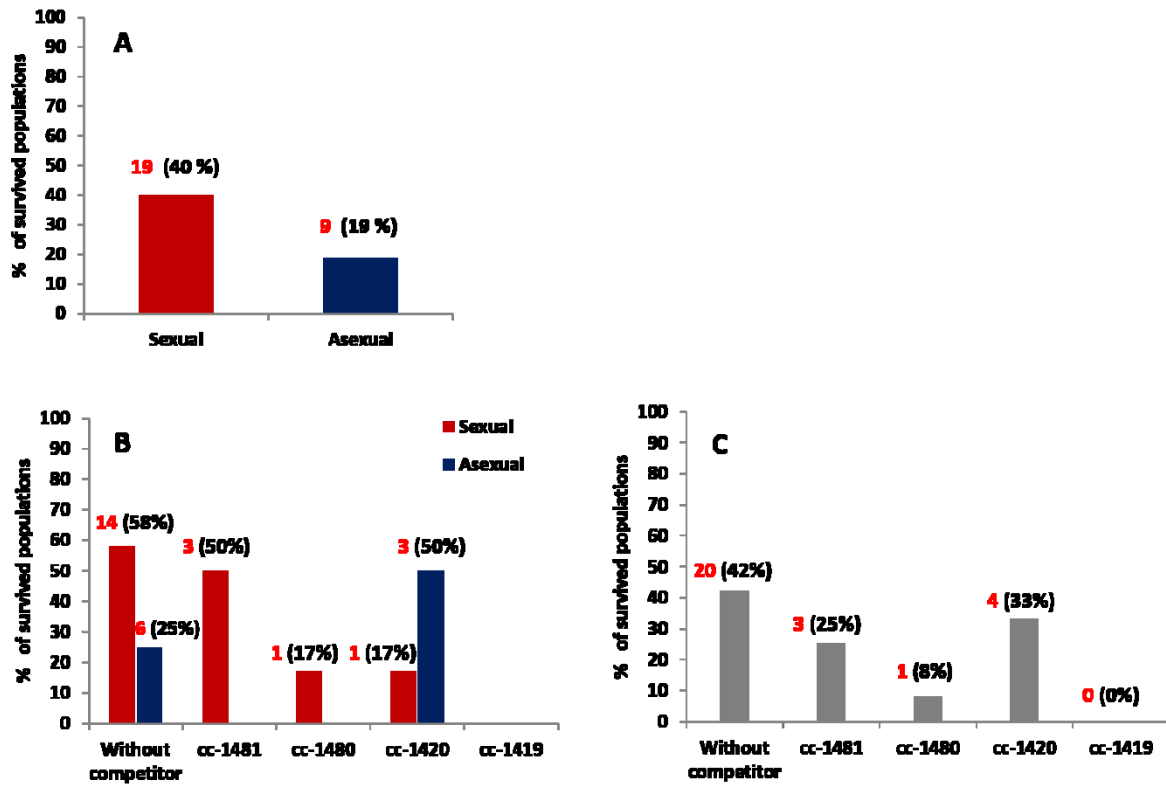
359 *Evolutionary rescue*

360 Overall, sex increased the probability of evolutionary rescue (Binary logistic
361 regression; $\chi^2 = 5.82$; $df = 1$; $P = 0.016$). Of the initial 48 populations for each mode of
362 reproduction, 19 sexual populations survived the lethal treatment (40%); in contrast, only 9
363 asexual populations (19%) avoided extinction (Figure 2A). Competition treatment affected
364 the probability of evolutionary rescue (Binary logistic regression; $\chi^2 = 10.3$; $df = 4$; $P = 0.005$).
365 Rescue was higher in the treatment with no competitor (42%) than in the 4 treatments
366 containing a competitor (33%, 25%, 8% and 0% populations survived propagated with cc-
367 1420, cc-1481, cc-1480 and cc-1419, respectively). The best fit was obtained by pooling the
368 three levels (corresponding to the three wild types of the competitor that caused most
369 extinctions) of 'competition treatment' factor. Hence, the final (minimum adequate) model
370 comprised the factor 'competition treatment' with three levels. The model simplification
371 suggested that the difference between the treatments with the competitor and no competition
372 treatment was significant for three of the competitor wild types: cc-1480, cc-1419 or cc-1481,
373 where rescue was substantially lower (Figure 2C).

374 However, whilst competition reduced evolutionary rescue in most of the treatments,
375 the benefit of sex was not affected by the presence of a competitor. Sexual populations had
376 the higher probability of evolutionary rescue both in the presence and absence of the
377 competitor (Figure 2B) and there was no evidence of an interaction between mode of
378 reproduction and competition treatment (Binary logistic regression; $\chi^2 = 8.0$; $df = 4$; $P =$
379 0.09).

380 *C. moewusii* had significantly higher probability of survival relative to *C. reinhardtii*,
381 since it went extinct in only 6 mixed populations (12%) (Binary logistic regression; $\chi^2 = 53.49$;
382 $df = 1$; $P < 0.0001$). The probability of survival of *C. moewusii* was contingent on the wild type
383 (Binary logistic regression; $\chi^2 = 10.08$; $df = 3$; $P = 0.02$): cc-1481 and cc-1420 have gone

384 extinct in 33% and 17% of mixed populations, respectively; no extinctions were recorded for
 385 cc-1480 and cc-1419 wild types. The probability of survival of *C. moewusii* did not depend on
 386 mode of reproduction of *C. reinhardtii* (Binary logistic regression; $\chi^2 = 0.74$; $df = 1$; $P = 0.39$).



387

388 **Figure 2** – Probability of evolutionary rescue for experimental populations of the focal species:
 389 for each mode of reproduction (A), with respect to both mode of reproduction and the
 390 competition treatment (B); with respect to the competition treatment (C); percentage of rescued
 391 populations per each treatment group is plotted on the Y axis; the number of survived populations per
 392 each treatment group is presented above each bar plot, with percentage of survived populations of the
 393 initial number of experimental populations in brackets; the initial number of experimental populations
 394 was 48 for each mode of reproduction and 48 for each treatment group propagated with or without the
 395 competitor, with equal number of populations (12) propagated with one of the four wild types of the
 396 competitor.

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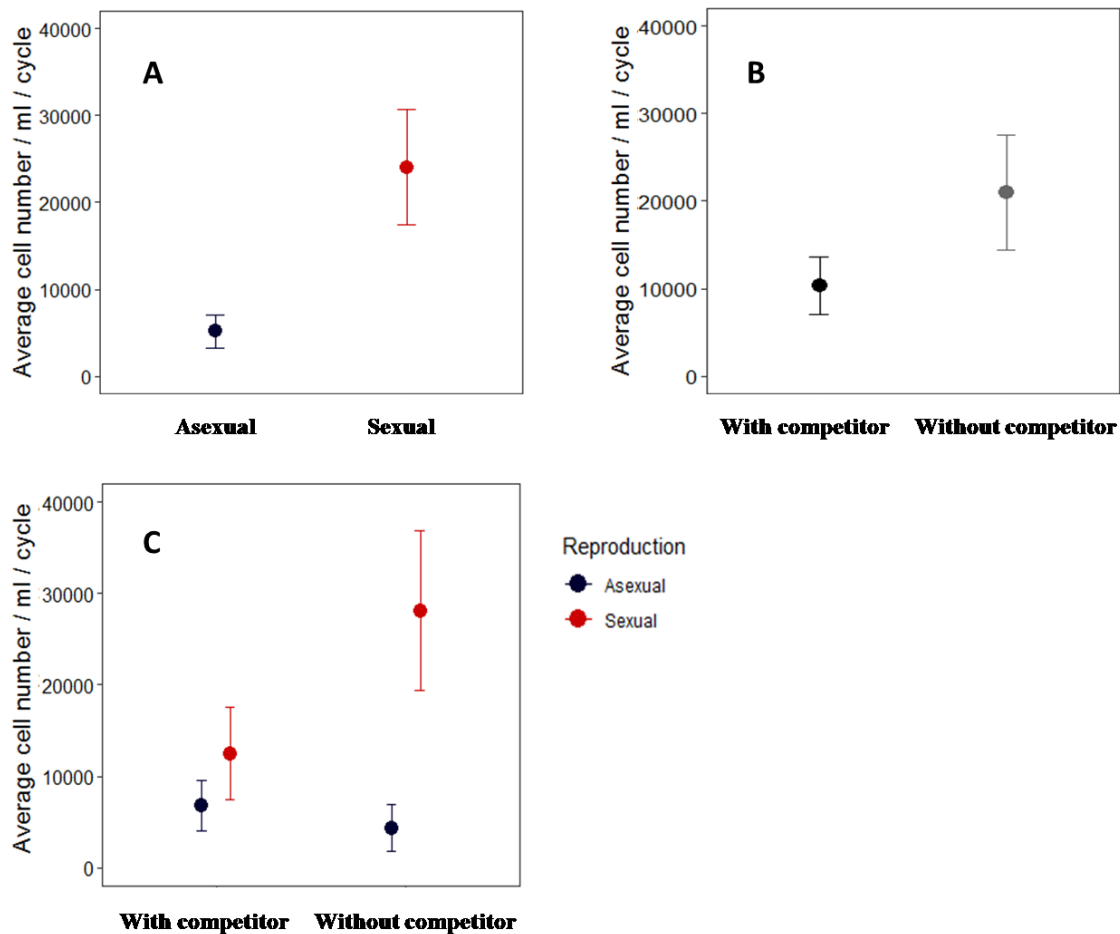
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400 ***Adaptedness of rescued populations***

401 In order to investigate the effect of mode of reproduction and competition treatment
402 on adaptedness of survived experimental populations of the focal species, we estimated
403 population size after each growth cycle by counting the colonies appeared on agar plates
404 after 4 days. Sex affected adaptedness of survived experimental populations (Two-way
405 ANOVA; $F_{1,27} = 5.09$; $P = 0.03$) (Figure 3A). Sexual populations which survived the lethal
406 treatment had more than four-fold advantage over asexual populations with respect to
407 population size. There was no evidence for any effect of the competition treatment on mean
408 population size of survived experimental populations (Two-way ANOVA; $F_{1,27} = 0.04$; $P =$
409 0.83) (Figure 3B). There was no interaction between mode of reproduction and competition
410 on adaptedness of survived populations (Two-way ANOVA; $F_{1,27} = 0.89$; $P = 0.35$). Survived
411 sexual populations had higher mean population size than asexual populations, irrespective
412 of the presence of the competitor (Figure 3C).

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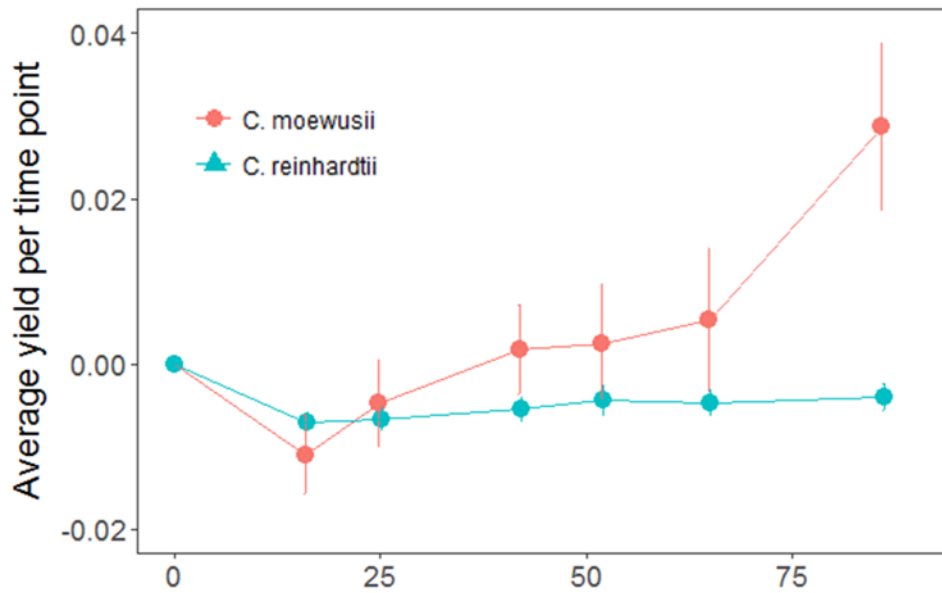
415 **Figure 3 - Mean population size of survived experimental populations of the focal species: for each**
 416 **mode of reproduction (A); with respect to competition treatment (B); with respect to both mode of**
 417 **reproduction and competition treatment (B); the bars represent standard error of the mean.**

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420 ***Assay of experimental species for high salt tolerance***

421 The growth rate of both species in the high salt environment, measured as the slope
 422 of the line representing mean change of a population size (yield) as a function of time, was
 423 significantly different (One-Way ANOVA; $F_{1,42} = 13.85$; $P = 0.0006$) (Figure 4). The average
 424 growth of *C. moewusii* wild type isolates was positive, despite considerable reduction in
 425 maximal yield in comparison to a benign medium (by 89%, data not shown). By contrast, the
 426 average growth of *C. reinhardtii* ancestral isolates was negative.



427

428 **Figure 4 - The average yield of *C. reinhardtii* ancestral isolates (blue) and *C. moewusii* wild type**
 429 **isolates (red) per each time point (h), measured in the high salt medium (Bold's supplemented**
 430 **with 15 g/l of salt); the bars represent standard error of the mean.**

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434 **5. Discussion**

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Previous studies have shown that sex can be beneficial when an environment deteriorates in a simple way (Lachapelle and Bell, 2012; Lachapelle, 2015). Our findings are consistent with these results, given that relative advantage of sexual over asexual populations (in the number of evolutionary rescue events) was 57% in an environment comprising a deteriorating abiotic component. To the best of our knowledge, this is the first study to examine how the advantageous effects of sex are affected by the presence of a competitor species. The results of our experiment indicate that sex remains beneficial in the presence of a competitor, given that sexual populations maintained the relative advantage in the number of evolutionary rescue events (40% higher than that of asexual populations). The advantage of sex is also reflected by the larger population sizes of sexual populations that avoided extinction, both in the presence and absence of the competitor. Furthermore, we

446 found no evidence that the size of the advantage of sex was affected by the presence of the
447 competitor, given the absence of interaction between mode of reproduction and competition
448 treatment.

449 The plausible explanation for the overall benefits of sex is a higher diversity of
450 favorable genotypes (with higher absolute fitness in stressful conditions) generated by sex
451 before the conditions have become lethal, which increased the efficiency of selection. These
452 genotypes may have risen in frequency and thus positively affected mean population size.
453 Since population size determines the supply of mutations beneficial in changing conditions
454 (Samani and Bell, 2010), sexual populations may have been more likely to adapt and survive
455 when conditions have become lethal.

456 We suggested three possible outcomes of interplay between sex and competition:
457 the costs of sex may be augmented by competition, resulting in overall disadvantage of sex
458 (the sign of the effect changes); sex may remain beneficial in the presence of competitor, but
459 the level of benefits may decline due to the negative effects of competition (the magnitude of
460 the effect decreases); the advantage of sex may increase with an increase of environmental
461 complexity (the magnitude of the effect increases). The lack of evidence of any interaction
462 between mode of reproduction and the competition treatment seemingly rules out all three of
463 these predictions. However, the actual difference in the relative survival of sexual
464 populations observed in our study was reasonably large (2.33 times the asexual survival
465 probability in the absence of the competitor and 1.66 times in the presence of the
466 competitor), suggesting there may have been differences that we did not have the power to
467 detect with our study. Thus, while our results allow us to reasonably confidently rule out
468 competition either substantially enhancing benefits of sex, or causing sex to become
469 detrimental, we cannot completely rule out competition causing a moderate reduction in
470 benefits of sex.

471 Whilst there is evidence that the advantage of sex increases with an increase of
472 complexity of novel abiotic environment (Kaltz and Bell, 2002; Luijckx et al., 2017) due to
473 faster assembling of multilocus genotypes, no such change of the effect was detected in the
474 presence of a competitor. This is surprising, since adaptation to the abiotic and biotic
475 component of novel environment may involve selection acting on different sets of traits
476 (Collins, 2011), which are likely to be controlled by multiple loci. The negative effect of
477 competition may manifest through reduction of population size, as demonstrated in the
478 experiments carried out in benign environments (Ayala 1969; Bengtsson 1989; Martin and
479 Martin, 2001). If the major negative effect of competition in our experiment was reduction of
480 population size of a focal species, the further increase of beneficial effect of sex with an
481 increase of environmental complexity (incorporation of the competitor into the environment)
482 may have been hampered by the limitation of favorable genotypes.

483 The overall effect of competition on the probability of evolutionary rescue was
484 negative. The focal species was driven to extinction in 83% of all the mixed populations,
485 compared to 58% probability of extinction in the absence of the competitor. Moreover,
486 negative effect of competition was consistent for all four wild types of the competitor (though
487 statistically significant for only three of them). Even though the probability of survival of *C.*
488 *moewusii* varied among the wild types, each wild type was more likely to survive than the
489 focal species and caused reduction of *C. reinhardtii* survival probability relative to the group
490 propagated in the absence of the competitor, which ranged from 9% (cc-1420) to 42% (cc-
491 1419). A lower sensitivity of the competitor to the elevated salt concentration, manifested
492 through a faster growth rate in the high salt medium is a plausible explanation for reduced
493 probability of survival of the focal species in the presence of *C. moewusii*.

494 For the interpretation of the results of our experiment, it is important to take into
495 account the possibility that survival of the populations of the focal species was due to
496 competitive exclusion of the competitor, without adaptive evolution. Given a higher
497 competitiveness of *C. moewusii* in the high salt medium (positive growth contrasted by

498 negative growth of *C. reinhardtii*), we rule out the possibility that survival of *C. reinhardtii* can
499 be attributed solely to competitive exclusion of *C. moewusii*. Since all the ancestral isolates
500 of our experimental populations showed negative growth in the high salt environment, any
501 recorded survival has to be the result of adaptive evolution (i.e. evolutionary rescue), which
502 may have then caused competitive exclusion of *C. moewusii*. We also rule out the possibility
503 that survival of *C. reinhardtii* populations was facilitated by the potential elimination of *C.*
504 *moewusii* from mixed populations in the early stages of experiment. Along with the
505 advantage of faster (and qualitatively different) growth, the competitor species rarely went
506 extinct throughout the course of the experiment (in only 12% of mixed populations), so we
507 can conclude that competition persisted for most populations throughout most of the
508 experiment.

509 Our experiment provided evidence that sex may be the beneficial strategy in the
510 presence of the competitor (though the magnitude of the effect may potentially decrease),
511 despite overall negative effect of competition. Given that competitive interactions are
512 widespread in nature, one of the explanations for the maintenance of sex by natural
513 selection may be the increased rate of adaptation of sexual populations both in the presence
514 and absence of competitors. An outstanding question is: will the effects of sex change if a
515 degree of complexity of environmental change further increases (with an increase of a
516 number of competitors)? A population size of adapting population may decline more
517 prominently in the presence of more competitors. With a lower supply of beneficial mutations
518 as a consequence, beneficial effect of sex may diminish. However, experimental evidence is
519 required.

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521 Will the effect of competition be altered by differences in sensitivity of competing
522 species to a stressor imposed on them? In our experiment, *C. moewusii* showed higher
523 degree of tolerance to elevated salt concentration than the focal species. However, the
524 negative effects of competition may be reduced if the difference between the competitors

525 with respect to maladaptedness (sensitivity) to a particular stressor is smaller. However, the
526 experiments with direct manipulation of this factor are required. Alternatively, the probability
527 of evolutionary rescue may be affected by other factors, such as ecological characteristics of
528 a competitor. Recent studies suggest that the negative effect of competition is proportional to
529 the niche overlap between competitors (Osmond and de Mazancourt, 2013). Two
530 ecologically separated competitors would potentially suffer a lower population decline than
531 the ones characterized by the similar patterns of resource use. Similarly, the phylogenetic
532 distance between competitors may indirectly influence the likelihood of evolutionary rescue,
533 under the hypothesis that the level of competition is directly proportional to relatedness
534 between competitors (Naughton et al., 2015). However, *C. moewusii* and *C. reinhardtii*
535 occupy different ecological niches, the former being a freshwater organism and the latter
536 terrestrial. Moreover, both species are on the opposite ends on the phylogenetic tree of
537 *Chlamydomonas* genus, thus being distantly related. This indicates that the effect of
538 competition on evolutionary rescue may remain negative, regardless of the identity of the
539 competitor. Whether this is the widespread pattern in nature, remains to be investigated.

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541 **Data accessibility**

542 Data will be available through the Dryad Digital Repository (datadryad.org).

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544 **Conflict of interest**

545 The authors declare no conflict of interest.

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