A single episode of sexual reproduction can prevent population extinction under multiple stressors

Yawako W. Kawaguchi[1], Masato Yamamichi[1,2,3,4,5*]

[1]Center for Frontier Research, National Institute of Genetics, 1111 Yata, Mishima, Shizuoka 411-8540, Japan

[2]Genetics Program, Graduate Institute for Advanced Studies, SOKENDAI, 1111 Yata, Mishima, Shizuoka 411-8540, Japan

[3]School of the Environment, The University of Queensland, St. Lucia, Brisbane, Queensland 4072, Australia

[4]Department of International Health and Medical Anthropology, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan

[5]Institute for Multidisciplinary Sciences, Yokohama National University, 79-5 Tokiwadai, Hodogaya, Yokohama, Kanagawa 240-8501, Japan

[*]Corresponding author: myamamichi@nig.ac.jp

ORCID

YWK: 0000-0002-0389-7744

MY: 0000-0003-2136-3399

Keywords

Adaptation, extinction, population biology, sex

Abstract

Recent studies have shown that organisms can adapt to changing environments through rapid contemporary evolution. Although intraspecific genetic variation is necessary for rapid evolution to occur, little is known how genetic variation has been produced and maintained before rapid evolution. Here we show that a single episode of sexual reproduction can produce a large amount of intraspecific trait variation that allows population growth in degraded environments by laboratory experiments of a green alga, *Closterium peracerosum–strigosum–littorale* complex. We observed population dynamics of the alga under multiple stressors and confirmed that high salinity and low pH decreased population growth rates. By comparing parental and their hybrid F_1 populations, we observed larger variation in population growth rates of F_1 populations (i.e., transgressive segregation) when pH was low. Interestingly, even when parental populations had negative growth rates, some F_1 populations showed positive growth rates in severe environmental conditions due to the large variation in population growth. By utilizing the recently obtained genomic information of the alga, we found greater enrichment of genes with copy number variations in terms related to pH stress than those to salt stress in a gene ontology (GO) enrichment analysis. Our results suggest that recombination and variation in the number of gene copies might produce large genetic variation in the F_1 generation. This will be an important step toward a better understanding of evolutionary rescue, where rapid evolution prevents population extinction in changing environments.

Introduction

Traditionally, evolutionary biologists tended to think that evolution is a slow process (Darwin 1859), but studies in the last quarter century have shown that organisms can flexibly adapt to changing environments through rapid contemporary evolution (Thompson 1998, Hairston et al. 2005, Schoener 2011, Hendry 2017, Sanderson et al. 2022). One of the most important implications of this phenomenon is that rapid evolution may prevent population extinction due to environmental changes, a process called "evolutionary rescue" (reviewed in Kinnison and Hairston 2007, Alexander et al. 2014, Carlson et al. 2014, Bell 2017). Previous studies have demonstrated that rapid evolution can prevent population extinction in severe environments by using mathematical modeling (e.g., Gomulkiewicz and Holt 1995, Orr and Unckless 2014, Uecker and Hermisson 2016, Orive et al. 2017), laboratory experiments (e.g., Bell and Gonzalez 2009, Lachapelle and Bell 2012, Lindsey et al. 2013), and field observations (e.g., Vander Wal et al. 2013, Isanta-Navarro et al. 2021).

It is well known, however, that evolution has no foresight and organisms cannot "prepare" for novel environmental changes in the future by maintaining genetic variation. How is genetic variation for rapid evolution produced and maintained in populations before the environmental changes? In addition to *de novo* mutations and immigration, classical studies in evolutionary biology have revealed that sexual reproduction is an important source for genetic variation (Williams 1975, Maynard Smith 1978, Bell 1982, Hartfield and Keightley 2012), and several experimental studies have indeed demonstrated that sexual reproduction can promote evolutionary rescue (Lachapelle and Bell 2012, Petkovic and Colegrave 2019, 2023). However, previous studies tended to compare experimental population dynamics of lineages with and without sexual reproduction and showed that sexual reproduction can increase the speed of adaptive evolution (Kaltz and Bell 2002, Goddard et al. 2005, Morran et al. 2009, Gray and Goddard 2012, Lachapelle and Bell 2012, Petkovic and Colegrave 2019, 2023). Thus, it was not clear how a single event of sexual reproduction can affect the amount of variation and the resultant rapid evolution. Colegrave et al. (2002) examined the effects of a single sexual episode on growth in various carbon sources, but they did not consider evolutionary rescue.

Here we show how a single episode of sexual reproduction can produce a large amount of trait variation and it makes population growth possible in degraded

environments using laboratory experiments of a unicellular green alga, *Closterium peracerosum–strigosum–littorale* complex (Fig. 1A). The alga is a freshwater species belonging to the Zygnematophyceae, a group most closely related to land plants (Wickett et al. 2014). It usually grows vegetatively (asexual reproduction), but it can also mate with another cell and form a zygospore that has desiccation tolerance (sexual reproduction: Fig. 1B). As we can easily induce sexual reproduction and obtain F_1 populations in laboratory experiments (Kawaguchi et al. 2023), we observed population dynamics of the alga under multiple stressors: salinity and acidity (low pH). Freshwater green algae go extinct under salinity stress (Lachapelle and Bell 2012, Petkovic and Colegrave 2019, 2023), and some species of the genus *Closterium* has been reported to prefer neutral or high pH water (Gough 1977, Renstrom 1979, Greenwood and Lowe 2006). By comparing parental and hybrid F_1 populations, we observed large variation in population growth rates of F_1 populations under low pH. This can be seen as an example of transgressive segregation, the formation of extreme phenotypes in hybrid populations (Rieseberg et al. 1999). Interestingly, even under severe environmental conditions when parental populations had negative growth rates, some F_1 populations showed positive growth rates. This suggests the potential importance of sexual reproduction for producing genetic variation within populations that can prevent population extinction in deteriorating environments. We further found greater enrichment of genes with copy number variations in terms related to pH stress than those to salt stress in a gene ontology (GO) enrichment analysis, and discuss the potential importance of gene copy number variation and recombination for producing genetic variation based on recently sequenced genomic information for the alga (Kawaguchi et al. 2023).

Materials and Methods

Base Population and Culture Conditions

Two strains of *C. psl.* complex, NIES-64 and NIES-65, were obtained from the National Institute for Environmental Studies (NIES), Environment Agency (Ibaraki, Japan). The strains are genetically close and sympatric (Kawaguchi et al. 2023). They are heterothallic strains and they can form zygospores between cells of two mating types: mating type plus (mt^+: NIES-65) and mating type minus (mt^-: NIES-64) (Tsuchikane

111 and Sekimoto 2019, Kawaguchi et al. 2023, Ohtaka and Sekimoto 2023). They
112 recognize each other through the sex pheromones and then form zygotes (Fig. 1B:
113 Sekimoto et al. 1994, Nojiri et al. 1995). Through sexual reproduction, haploid parental
114 populations produce diploid zygospores, and then haploid offspring (F_1) populations
115 arise via meiosis and recombination (Fig. 1B).
116 The base population used for the experiments was cultured in a nitrogen-
117 supplemented medium (CA medium). The CA medium contains the following reagents
118 in 99.9 mL of distilled water: 2 mg of calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$),
119 10 mg of potassium nitrate (KNO_3), 5 mg of ammonium nitrate (NH_4NO_3), 3 mg of
120 sodium beta-glycerophosphate pentahydrate (β-Na2glycerophosphate · $5H_2O$), 2 mg of
121 magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), 0.01 µg of vitamin B_{12}, 0.01 µg of
122 biotin, 1 µg of thiamine HCl, 0.1 mL of PIV metal solution, 0.1 mL of Fe (as EDTA; 1:1
123 molar), and 40 mg of HEPES. The PIV metal solution contains the following reagents in
124 100 mL of distilled water: 100 mg $Na_2EDTA \cdot 2H_2O$, 19.6 mg $FeCl_3 \cdot 6H_2O$, 3.6 mg
125 $MnCl_2 \cdot 4H_2O$, 1.04 mg $ZnCl_2$, 0.4 mg $CoCl_2 \cdot 6H_2O$ and 0.25 mg $Na_2MoO_4 \cdot 2H_2O$ (pH
126 7.2, 0% NaCl; Kasai et al. 2004). The culture conditions were maintained at 22 °C
127 under a 14 h light/10 h dark cycle. The light intensity was about 40 µmol photons m^{-2} s^{-1}.
128
129 ***Establishment of F_1 Populations***
130 We grew cells from the parental strains, NIES-64 and NIES-65, vegetably for 10 days
131 and then they were washed two times with a nitrogen-depleted mating-inducing medium
132 (MI medium; Ichimura 1971). The MI medium contains the following reagents in 99.7
133 mL of distilled water: 4 mg of $MgSO_4 \cdot 7H_2O$, 5 mg of β-Na_2glycerophosphate · $5H_2O$,
134 10 mg of $CaCl_2 \cdot 2H_2O$, 0.01 µg of vitamin B_{12}, 0.01 µg of Biotin, 1 µg of thiamine HCl,
135 0.3 mL of PIV metals, and 50 mg of Tris (hydroxymethyl) aminomethane. Subsequently,
136 the two strains were mixed and incubated in the MI medium at a concentration of
137 10,000 cells/ml for each strain. This mixture was exposed to 24 h continuous light for
138 10 days. The zygotes were then kept in the dark conditions for a week until they were
139 completely dried out (note that they have desiccation tolerance). After a week, we added
140 the CA medium to induce germination of the zygospores. Through these processes, we
141 established eight F_1 populations in total. Populations F_1-A through F_1-E germinated
142 from 1,000 zygospores each, whereas F_1-F germinated from 100 zygospores and F_1-G
143 and F_1-H germinated from 10,000 zygospores.

144

145 ***Culturing of Populations***

146 The populations of NIES-64, NIES-65, and eight F_1 populations were cultured in six-
147 well plates at a density of 2,500 cells per 5 mL CA medium (Fig. S1). The culture
148 conditions varied across combinations of NaCl concentrations (0, 0. 05, 0.1, and 0.5%)
149 and pH levels (6.2, 7.2, and 8.2), resulting in a total of 12 different conditions. For
150 reference, 0.5% NaCl corresponds to 5g/L NaCl and ca. 0.0856 M whereas seawater is
151 about 3% NaCl (ca. 0.5 M). Each condition was replicated five times.

152

153 ***Measurement of Growth Rate***

154 To calculate the population growth rate, algal biomass was measured on the first day
155 (day 0; the day the cells were introduced to each medium), as well as on days 1, 2, and 6,
156 using a Multiskan SkyHigh TCD (Thermo) at an absorbance of 684 nm as like previous
157 experimental studies on *Chlamydomonas* (Lachapelle and Bell 2012, Petkovic and
158 Colegrave 2019, 2023). As the pigment content variation of a cell may bias the
159 estimation of algal biomass, we used a wavelength where absorbance by the pigment is
160 small (Griffiths et al. 2011). We also checked the correlation between the absorbance
161 and the number of cells (Fig. S2). Before every measurement, the cells were agitated by
162 pipetting. To eliminate the effects of the background, the absorbance values of media
163 without cells were subtracted from all measurements.

164 The biomass dynamics was modeled using nonlinear least-squares fitting with
165 the minpack.lm package (Elzhov et al. 2016) in R (version 4.3.2) (R Core Team 2023)
166 as follows:

167

168 $$N_t = N_0 e^{rt}, \quad (1)$$

169

170 where N_t represents the algal biomass (absorbance values) on day t, r is the biomass
171 growth rate, t denotes the number of days, and N_0 is the algal biomass on day 0.
172 Hereafter we use "population growth" to represent biomass growth dynamics (Fig. S3).

173 To investigate whether the variation in growth rates in the F_1 populations is
174 significantly larger than those expected from the parental (NIES-64 and NIES-65)
175 populations, we calculated a mean and a standard deviation (SD) in population growth
176 rates of the two parental populations. Then we sampled F_1 individuals from a normal

177 distribution with the calculated mean and SD. This is an expected distribution of F_1
178 growth rates, and we calculated a SD of the distribution. The simulation was repeated
179 10,000 times to construct a null distribution of the SDs. We then examined whether the
180 observed SDs of the F_1 population significantly deviated from these simulated
181 distributions (whether $p < 0.01$ or not).
182
183
184 **Results**
185 We first analyzed how salinity and low pH affect population growth rates, *r*. To simplify
186 our analyses, we focused on conditions with 0.05%, 0.1%, and 0.5% NaCl and pH 6.2
187 and 7.2 in the main text (Fig. 2). See Fig. S3 and S4 for the entire dataset including
188 conditions with 0% NaCl and/or pH 8.2. We found that both stresses decreased
189 population growth rates significantly (two-way ANOVA: salinity; $F_{2,291} = 101$, $p <$
190 0.001, pH; $F_{1,291} = 46.5$, $p < 0.001$, Fig. 2, Table S1). In addition, the effects of stressors
191 were not additive (salinity × pH interaction; $F_{2,291} = 7.24$, $p < 0.001$, Fig. 2, Table S1),
192 indicating the importance of considering two stressors simultaneously. The results from
193 Fig. 2 were consistent with those based on the entire dataset (Table S2). As expected,
194 we found that combining two types of stressors can further decrease population growth
195 rates: for example, most populations showed positive growth rates in the condition with
196 0.05% NaCl and pH 6.2 (Fig. 2A, S4B) as well as that with 0.1% NaCl and pH 7.2 (Fig.
197 2E, S4G), but many of them showed negative growth rates in the condition with 0.1%
198 NaCl and pH 6.2 (Fig. 2B, S4C). Growth responses of the two parental populations to
199 stressors can be sometimes similar (Fig. 2B-C), but sometimes different (Fig. 2E).
200 Under the 0.1% NaCl and pH 7.2 condition (Fig. 2E, S4G), the NIES-64 strain showed
201 a significantly higher growth rate than the NIES-65 strain (Student's *t*-test: *t*-value =
202 3.74, d.f. = 7, $p = 0.00726$).
203 Under the stressful conditions, some F_1 populations exhibited larger variation
204 in growth rates compared with the parental ones (Fig. 2). Specifically, under conditions
205 of pH 6.2 with 0%, 0.05%, 0.1%, and 0.5% NaCl; pH 7.2 with 0%; and pH 8.2 with
206 0.5% NaCl, the F_1 populations showed significantly greater standard deviations in
207 growth rates than those expected from parental populations ($p < 0.01$, Fig. 2A-C and
208 S4A-E and L, see Materials and Methods section for details). For example, in the
209 condition with 0.05% NaCl and pH 6.2, the two parental populations showed very

210 similar positive growth rates whereas the F_1 populations exhibited large variation of
211 growth rates: the variation is so large that some of them are even negative (Fig. 2A,
212 S4B). Furthermore, some F_1 populations demonstrated positive growth rates even under
213 severe stress conditions where the parental populations showed negative growth rates
214 (0.1% NaCl and pH 6.2, Fig. 2B, S4C). Even in the severest condition (0.5% NaCl and
215 pH 6.2), some F_1 populations showed positive growth rates (Fig. 2C, S4D). We also
216 found that F_1 populations germinated from larger number of zygospores exhibited
217 higher growth rates in two conditions (Fig. S5D, S5F), but it is not always the case (see
218 Fig. S5 for details).
219
220
221 **Discussion**
222 Sexual reproduction has been a central enigma in evolutionary biology because of the
223 many costs of sex (Williams 1975, Maynard Smith 1978, Bell 1982, Hartfield and
224 Keightley 2012, Lehtonen et al. 2012). Recent studies have shown that sexual
225 reproduction is important for organisms to adapt to rapidly changing environments
226 (Colegrave 2002, Kaltz and Bell 2002, Goddard et al. 2005, Morran et al. 2009, Becks
227 and Agrawal 2010, Gray and Goddard 2012) and for preventing population extinction
228 (Lachapelle and Bell 2012, Petkovic and Colegrave 2019, 2023), but it was not clear
229 whether a single episode of sexual reproduction can produce large amount of genetic
230 variation. By using the unicellular green alga, *Closterium peracerosum–strigosum–*
231 *littorale* complex, we have shown large variation in population growth rates of F_1
232 populations (Fig. 2, S4). Even when parental populations show negative growth rates,
233 some F_1 populations show positive growth rates in severe environmental conditions
234 (e.g., the condition with 0.1% NaCl and pH 6.2, Fig. 2B, S4C). Our results indicate that,
235 although asexual reproduction is more efficient in population growth, sexual
236 reproduction is important for growth in stressful environments and for evolutionary
237 rescue.
238 Theory suggests that recombination due to sexual reproduction has two
239 antagonistic effects on evolutionary rescue: it generates and breaks up favorable gene
240 combinations (Uecker and Hermisson 2016, Orive et al. 2017). The former is related to
241 the Fisher-Muller hypothesis where sexual recombination can combine beneficial alleles
242 into the same genome (Fisher 1930, Muller 1932). Our results are consistent with the

243 two classical ideas: sexual reproduction tended to increase the variance in population
244 growth rates, and it resulted in positive (or negative) growth of some F_1 populations
245 even when their parental populations showed negative (or positive) growth (Fig. 2, S4).
246 Genome information recently obtained for this algal species (*C. psl.* complex) has
247 revealed that approximately 30% of the genes in the parental strains showed copy
248 number differentiation (Kawaguchi et al. 2023). Variation in the number of gene copies
249 may produce large variation in the F_1 generation through recombination (Hastings et al.
250 2009). Thus, it will be interesting to carefully consider how genetic architecture,
251 including structural variation, affects evolutionary rescue in future studies
252 (Gomulkiewicz et al. 2010, Yamamichi 2022).
253 When we regard population growth rate as a quantitative trait, it is possible to
254 consider our results as an example of transgressive segregation (Rieseberg et al. 1999).
255 Previous studies tended to focus on its effects on macroevolution such as speciation and
256 adaptive radiation (e.g., Kagawa and Takimoto 2018), but our results suggest that it may
257 affect rapid contemporary evolution as well as evolutionary rescue. Kawaguchi et al.
258 (2023) estimated the genome sizes of the F_1 populations between NIES-64 and NIES-65
259 and found that they were intermediate values between those of their parental
260 populations (Fig. 3a of Kawaguchi et al. 2023). This is consistent with the results on
261 population growth rates in, for example, Fig. 2E, but not consistent with the results
262 under low pH (Fig. 2A-C). Thus, it will be important to explore causes of the difference
263 between the estimated genome size and population growth rates in future studies. In this
264 context, it will be helpful to conduct linkage mapping to understand which genes are
265 responsible for population growth rates. Green algae are suitable as they grow as
266 haploids, and thus it should be easier to detect genes responsible for a quantitative trait
267 without considering allelic dominance.
268 We observed that the green alga grew very well in high pH medium (when pH
269 was 8.2, Fig. S3, S4), which is consistent with previous studies on some species of the
270 genus *Closterium* (Gough 1977, Renstrom 1979, Greenwood and Lowe 2006). On the
271 other hand, low pH reduced growth rates significantly as expected (when pH was 6.2,
272 Fig. 2, S3, S4). Interestingly, the F_1 populations tended to have larger variation in
273 growth rates in low pH environments (Fig. 2A-C). This may be seen as an example of
274 cryptic genetic variation, which has little or no effect on phenotype normally but
275 generates phenotypic variation under novel conditions (Paaby and Rockman 2014).

276 Previous studies on *Chlamydomonas* species have suggested that genes encoding heat-
277 shock proteins and plasma membrane H^+-ATPase were attributable to adaptation to low
278 pH environments (Gerloff-Elias et al. 2006, Hirooka et al. 2017). In the genus
279 *Klebsormidium*, belonging to streptophyte algae (as like *Closterium*), it has been
280 demonstrated that the ability to adapt to low pH conditions has evolved multiple times
281 independently (Škaloud et al. 2014). These previous studies, combined with our
282 findings, imply that adaptation to low pH environments may be related to cryptic
283 genetic variation that can promote rapid adaptation.
284 Under high salt stress, we also observed a notable reduction in population
285 growth rate (Fig. 2, S3, S4). Salt is known to impose osmotic and oxidative stresses by
286 disrupting the homeostasis of ions and inhibits growth by decreasing the rates of
287 photosynthesis (Husic and Tolbert 1986, Neelam and Subramanyam 2013) and promote
288 population extinction in *Chlamydomonas* (Lachapelle and Bell 2012, Petkovic and
289 Colegrave 2019, 2023). However, unlike low pH, salt stress alone did not result in
290 increased variation in growth rates of F_1 populations (Fig. 2D-F). Our additional
291 analyses suggest that the large variation observed in the F_1 populations under low pH
292 may be attributed to copy number variation between parental strains. Previous studies
293 have reported that there was a difference in chromosome numbers between the parental
294 strains NIES-64 and NIES-65, and approximately 30% of genes exhibited variations in
295 copy number (Kawaguchi et al. 2023, Tsuchikane et al. in press). Following the
296 methodology of Kawaguchi et al. (2023), our Gene Ontology (GO) enrichment analysis
297 revealed greater enrichment of genes with copy number variations in terms related to
298 pH stress than those to salt stress (see Fig. S6 for more details). Notably, the term
299 "regulation of pH" (GO:0006885) showed significant enrichment ($p = 0.0339$). It
300 suggests that the copy number variation contributes to the greater variance observed in
301 F_1 populations under low pH conditions. Further analysis comparing genes involving in
302 pH and salt stress responses would be valuable, providing insights into how genetic
303 structures, environmental stressors, and recombination contribute to transgressive
304 segregation and promote evolutionary rescue.
305 While previous studies tended to focus on a single environmental factor to
306 understand its deteriorating effect on population growth and evolutionary rescue
307 (Kinnison and Hairston 2007, Alexander et al. 2014, Carlson et al. 2014, Bell 2017),
308 recent studies proposed that multiple stressors are simultaneously affecting ecosystem

dynamics in the Anthropocene (Orr et al. 2020). Therefore, it will be important to consider interacting multiple stressors in future studies on evolutionary rescue to deepen our understanding on conservation of endangered species as well as management of target species for hunting and fisheries. For archiving this goal, it will be essential to utilize genome information to reveal the genetic architecture underlying adaptation. If there are two or more stresses, more loci will affect phenotypic adaptation to the degraded environments, and it will further make sexual recombination important for producing combinations better adapted to the environmental changes.

Author contributions

Conceptualization: MY and YWK; Experiments and data analyses: YWK; Funding acquisition: MY; Writing-original draft: MY; Writing-reviewing and editing: MY and YWK.

Acknowledgments

C. psl. complex (NIES-64 and NIES-65) were provided by NIES through the National BioResource Project (NBRP) of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. We thank T. Tsuchimatsu, M. Aono, S. Shibasaki, and N. G. Hairston Jr. for their helpful comments and laboratory works.

Funding

YWK is supported by Japan Society for the Promotion of Science (JSPS) Grants-in-Aid for Scientific Research (KAKENHI) JP20J20877 and the National Institute of Genetics (NIG) Postdoctoral Research Fellowship. MY is supported by JSPS KAKENHI JP19K16223, JP20KK0169, JP21H02560, JP22H02688, and JP22H04983, Japan Science and Technology Agency (JST) Core Research for Evolutional Science and Technology (CREST) JPMJCR23N5, and Australian Research Council (ARC) Discovery Project DP220102040.

Conflict of interest

Authors declare that they have no competing interests.

References

Alexander, H. K., G. Martin, O. Y. Martin, and S. Bonhoeffer. 2014. Evolutionary rescue: linking theory for conservation and medicine. Evolutionary Applications **7**:1161-1179.

Becks, L., and A. F. Agrawal. 2010. Higher rates of sex evolve in spatially heterogeneous environments. Nature **468**:89-92.

Bell, G. 1982. The Masterpiece of Nature: The Evolution and Genetics of Sexuality. University of California Press, Berkeley, CA.

Bell, G. 2017. Evolutionary rescue. Annual Review of Ecology, Evolution, and Systematics **48**:605-627.

Bell, G., and A. Gonzalez. 2009. Evolutionary rescue can prevent extinction following environmental change. Ecology Letters **12**:942-948.

Carlson, S. M., C. J. Cunningham, and P. A. H. Westley. 2014. Evolutionary rescue in a changing world. Trends in Ecology & Evolution **29**:521-530.

Colegrave, N. 2002. Sex releases the speed limit on evolution. Nature **420**:664-666.

Colegrave, N., O. Kaltz, and G. Bell. 2002. The ecology and genetics of fitness in *Chlamydomonas*. VIII. The dynamics of adaptation to novel environments after a single episode of sex. Evolution **56**:14-21.

Darwin, C. 1859. On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life. John Murray, London.

Elzhov, T. V., K. M. Mullen, A.-N. Spiess, and B. Bolker. 2016. minpack.lm: R interface to the Levenberg-Marquardt nonlinear least-squares algorithm found in MINPACK, plus support for bounds. The Comprehensive R Archive Network.

Fisher, R. A. 1930. The Genetical Theory of Natural Selection. The Clarendon Press, Oxford, U.K.

Gerloff-Elias, A., D. Barua, A. Mölich, and E. Spijkerman. 2006. Temperature-and pH-dependent accumulation of heat-shock proteins in the acidophilic green alga *Chlamydomonas acidophila*. FEMS Microbiology Ecology **56**:345-354.

Goddard, M. R., H. C. J. Godfray, and A. Burt. 2005. Sex increases the efficacy of natural selection in experimental yeast populations. Nature **434**:636-640.

Gomulkiewicz, R., and R. D. Holt. 1995. When does evolution by natural selection prevent extinction? Evolution **49**:201-207.

375 Gomulkiewicz, R., R. D. Holt, M. Barfield, and S. L. Nuismer. 2010. Genetics,
376 adaptation, and invasion in harsh environments. Evolutionary Applications **3**:97-
377 108.
378 Gough, S. B. 1977. The growth of selected desmid (Desmidiales, Chlorophyta) taxa at
379 different calcium and pH levels. American Journal of Botany **64**:1297-1299.
380 Gray, J. C., and M. R. Goddard. 2012. Sex enhances adaptation by unlinking beneficial
381 from detrimental mutations in experimental yeast populations. BMC
382 Evolutionary Biology **12**:43.
383 Greenwood, J. L., and R. L. Lowe. 2006. The effects of pH on a periphyton community
384 in an acidic wetland, USA. Hydrobiologia **561**:71-82.
385 Griffiths, M. J., C. Garcin, R. P. van Hille, and S. T. L. Harrison. 2011. Interference by
386 pigment in the estimation of microalgal biomass concentration by optical density.
387 Journal of Microbiological Methods **85**:119-123.
388 Hairston, N. G., Jr., S. P. Ellner, M. A. Geber, T. Yoshida, and J. A. Fox. 2005. Rapid
389 evolution and the convergence of ecological and evolutionary time. Ecology
390 Letters **8**:1114-1127.
391 Hartfield, M., and P. D. Keightley. 2012. Current hypotheses for the evolution of sex
392 and recombination. Integrative zoology **7**:192-209.
393 Hastings, P. J., J. R. Lupski, S. M. Rosenberg, and G. Ira. 2009. Mechanisms of change
394 in gene copy number. Nature Reviews Genetics **10**:551-564.
395 Hendry, A. P. 2017. Eco-evolutionary Dynamics. Princeton University Press, Princeton,
396 NJ.
397 Hirooka, S., Y. Hirose, Y. Kanesaki, S. Higuchi, T. Fujiwara, R. Onuma, A. Era, R.
398 Ohbayashi, A. Uzuka, H. Nozaki, H. Yoshikawa, and S.-y. Miyagishima. 2017.
399 Acidophilic green algal genome provides insights into adaptation to an acidic
400 environment. Proceedings of the National Academy of Sciences of the United
401 States of America **114**:E8304-E8313.
402 Husic, H. D., and N. E. Tolbert. 1986. Effect of osmotic stress on carbon metabolism in
403 *Chlamydomonas reinhardtii*: accumulation of glycerol as an osmoregulatory
404 solute. Plant Physiology **82**:594-596.
405 Ichimura, T. 1971. Sexual cell division and conjugation-papilla formation in sexual
406 reproduction of *Closterium strigosum*. Pages 208-214 *in* Proceedings of the 7th
407 International Seaweed Symposium. The University of Tokyo Press, Tokyo.

Isanta-Navarro, J., N. G. Hairston, Jr., J. Beninde, A. Meyer, D. Straile, M. Möst, and D. Martin-Creuzburg. 2021. Reversed evolution of grazer resistance to cyanobacteria. Nature Communications **12**:1945.

Kagawa, K., and G. Takimoto. 2018. Hybridization can promote adaptive radiation by means of transgressive segregation. Ecology Letters **21**:264-274.

Kaltz, O., and G. Bell. 2002. The ecology and genetics of fitness in *Chlamydomonas*. XII. Repeated sexual episodes increase rates of adaptation to novel environments. Evolution **56**:1743-1753.

Kasai, F., M. Kawachi, M. Erata, and M. M. Watanabe. 2004. NIES-Collection list of strains seventh edition microalgae and protozoa. Res. Rep. NIES **182**:1-257.

Kawaguchi, Y. W., Y. Tsuchikane, K. Tanaka, T. Taji, Y. Suzuki, A. Toyoda, M. Ito, Y. Watano, T. Nishiyama, and H. Sekimoto. 2023. Extensive copy number variation explains genome size variation in the unicellular Zygnematophycean alga, *Closterium peracerosum–strigosum–littorale* complex. Genome Biology and Evolution **15**:evad115.

Kinnison, M. T., and N. G. Hairston, Jr. 2007. Eco-evolutionary conservation biology: contemporary evolution and the dynamics of persistence. Functional Ecology **21**:444-454.

Lachapelle, J., and G. Bell. 2012. Evolutionary rescue of sexual and asexual populations in a deteriorating environment. Evolution **66**:3508-3518.

Lehtonen, J., M. D. Jennions, and H. Kokko. 2012. The many costs of sex. Trends in Ecology & Evolution **27**:172-178.

Lindsey, H. A., J. Gallie, S. Taylor, and B. Kerr. 2013. Evolutionary rescue from extinction is contingent on a lower rate of environmental change. Nature **494**:463-467.

Maynard Smith, J. 1978. The Evolution of Sex. Cambridge University Press, Cambridge, U.K.

Morran, L. T., M. D. Parmenter, and P. C. Phillips. 2009. Mutation load and rapid adaptation favour outcrossing over self-fertilization. Nature **462**:350-352.

Muller, H. J. 1932. Some genetic aspects of sex. The American Naturalist **66**:118-138.

Neelam, S., and R. Subramanyam. 2013. Alteration of photochemistry and protein degradation of photosystem II from *Chlamydomonas reinhardtii* under high salt grown cells. Journal of Photochemistry and Photobiology B: Biology **124**:63-70.

Nojiri, T., T. Fujii, and H. Sekimoto. 1995. Purification and characterization of a novel sex pheromone that induces the release of another sex pheromone during sexual reproduction of the heterothallic *Closterium peracerosum-strigosum-littorale* complex. Plant and cell physiology **36**:79-84.

Ohtaka, K., and H. Sekimoto. 2023. Zygnematophycean algae: Possible models for cellular and evolutionary biology. Pages 59-68 *in* Seminars in Cell & Developmental Biology. Elsevier.

Orive, M. E., M. Barfield, C. Fernandez, and R. D. Holt. 2017. Effects of clonal reproduction on evolutionary lag and evolutionary rescue. The American Naturalist **190**:469-490.

Orr, H. A., and R. L. Unckless. 2014. The population genetics of evolutionary rescue. PLOS Genetics **10**:e1004551.

Orr, J. A., R. D. Vinebrooke, M. C. Jackson, K. J. Kroeker, R. L. Kordas, C. Mantyka-Pringle, P. J. Van den Brink, F. De Laender, R. Stoks, M. Holmstrup, C. D. Matthaei, W. A. Monk, M. R. Penk, S. Leuzinger, R. B. Schäfer, and J. J. Piggott. 2020. Towards a unified study of multiple stressors: divisions and common goals across research disciplines. Proceedings of the Royal Society B: Biological Sciences **287**:20200421.

Paaby, A. B., and M. V. Rockman. 2014. Cryptic genetic variation: evolution's hidden substrate. Nature Reviews Genetics **15**:247-258.

Petkovic, N., and N. Colegrave. 2019. Sex increases the probability of evolutionary rescue in the presence of a competitor. Journal of Evolutionary Biology **32**:1252-1261.

Petkovic, N., and N. Colegrave. 2023. The effects of sex on extinction dynamics of *Chlamydomonas reinhardtii* depend on the rate of environmental change. Journal of Evolutionary Biology **36**:1783-1795.

R Core Team. 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Renstrom, D. B. 1979. Culture and growth of *Closterium ehrenbergii* (Desmidiaceae). Portland State University.

Rieseberg, L. H., M. A. Archer, and R. K. Wayne. 1999. Transgressive segregation, adaptation and speciation. Heredity **83**:363-372.

Sanderson, S., M.-O. Beausoleil, R. E. O'Dea, Z. T. Wood, C. Correa, V. Frankel, L. D.

Gorné, G. E. Haines, M. T. Kinnison, K. B. Oke, F. Pelletier, F. Pérez-Jvostov, W. D. Reyes-Corral, Y. Ritchot, F. Sorbara, K. M. Gotanda, and A. P. Hendry. 2022. The pace of modern life, revisited. Molecular Ecology **31**:1028-1043.

Schoener, T. W. 2011. The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. science **331**:426-429.

Sekimoto, H., Y. Sone, and T. Fujii. 1994. Regulation of expression of the genes for a sex pheromone by an inducer of the sex pheromone in the *Closterium peracerosum-strigosum-littorale* complex. Planta **193**:137-144.

Škaloud, P., A. Lukešová, V. Malavasi, D. Ryšánek, K. Hrčková, and F. Rindi. 2014. Molecular evidence for the polyphyletic origin of low pH adaptation in the genus *Klebsormidium* (Klebsormidiophyceae, Streptophyta). Plant Ecology and Evolution **147**:333-345.

Thompson, J. N. 1998. Rapid evolution as an ecological process. Trends in Ecology & Evolution **13**:329-332.

Tsuchikane, Y., and H. Sekimoto. 2019. The genus *Closterium*, a new model organism to study sexual reproduction in streptophytes. New Phytologist **221**:99-104.

Tsuchikane, Y., M. Watanabe, Y. W. Kawaguchi, K. Uehara, T. Nishiyama, H. Sekimoto, and T. Tsuchimatsu. in press. Diversity of genome size and chromosome number in homothallic and heterothallic strains of the *Closterium peracerosum–strigosum–littorale* complex (Desmidiales, Zygnematophyceae, Streptophyta). Journal of Phycology.

Uecker, H., and J. Hermisson. 2016. The role of recombination in evolutionary rescue. Genetics **202**:721-732.

Vander Wal, E., D. Garant, M. Festa-Bianchet, and F. Pelletier. 2013. Evolutionary rescue in vertebrates: evidence, applications and uncertainty. Philosophical Transactions of the Royal Society B: Biological Sciences **368**:20120090.

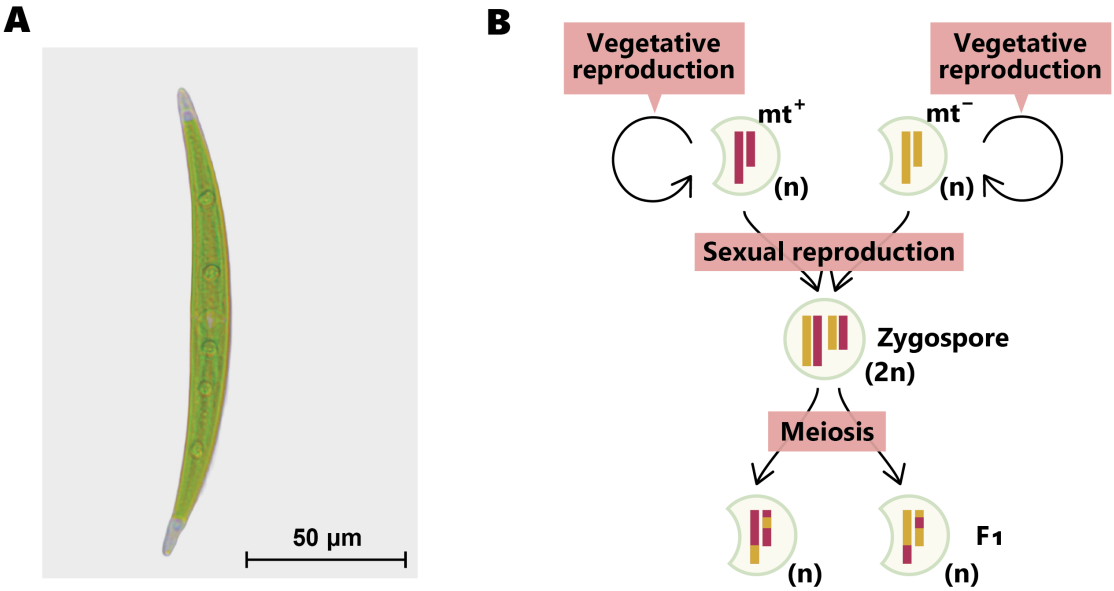
Wickett, N. J., S. Mirarab, N. Nguyen, T. Warnow, E. Carpenter, N. Matasci, S. Ayyampalayam, M. S. Barker, J. G. Burleigh, M. A. Gitzendanner, B. R. Ruhfel, E. Wafula, J. P. Der, S. W. Graham, S. Mathews, M. Melkonian, D. E. Soltis, P. S. Soltis, N. W. Miles, C. J. Rothfels, L. Pokorny, A. J. Shaw, L. DeGironimo, D. W. Stevenson, B. Surek, J. C. Villarreal, B. Roure, H. Philippe, C. W. dePamphilis, T. Chen, M. K. Deyholos, R. S. Baucom, T. M. Kutchan, M. M. Augustin, J. Wang, Y. Zhang, Z. Tian, Z. Yan, X. Wu, X. Sun, G. K.-S. Wong,

and J. Leebens-Mack. 2014. Phylotranscriptomic analysis of the origin and early diversification of land plants. Proceedings of the National Academy of Sciences of the United States of America **111**:E4859-E4868.

Williams, G. C. 1975. Sex and Evolution. Princeton University Press, Princeton, NJ.

Yamamichi, M. 2022. How does genetic architecture affect eco-evolutionary dynamics? A theoretical perspective. Philosophical Transactions of the Royal Society B: Biological Sciences **377**:20200504.

515 **Figures**



516

517 **Fig. 1. Green alga, *Closterium peracerosum–strigosum–littorale* complex, and its life**
518 **cycle.**
519 (A) A cell of *C. psl.* complex (NIES-65). (B) The life cycle of *C. psl.* complex, with
520 mating types "mt^+" (mating type plus) and "mt^-" (mating type minus). Chromosomes
521 from parental strains, mt^- and mt^+, are colored by orange and red, respectively. The
522 symbols "n" and "2n" denote haploid and diploid cells, respectively. Haploid cells
523 proliferate via vegetative reproduction, whereas sexual reproduction between different
524 mating types produces diploid zygospores. Then, haploid cells (F_1) were produced from
525 the diploid zygospores through meiosis.

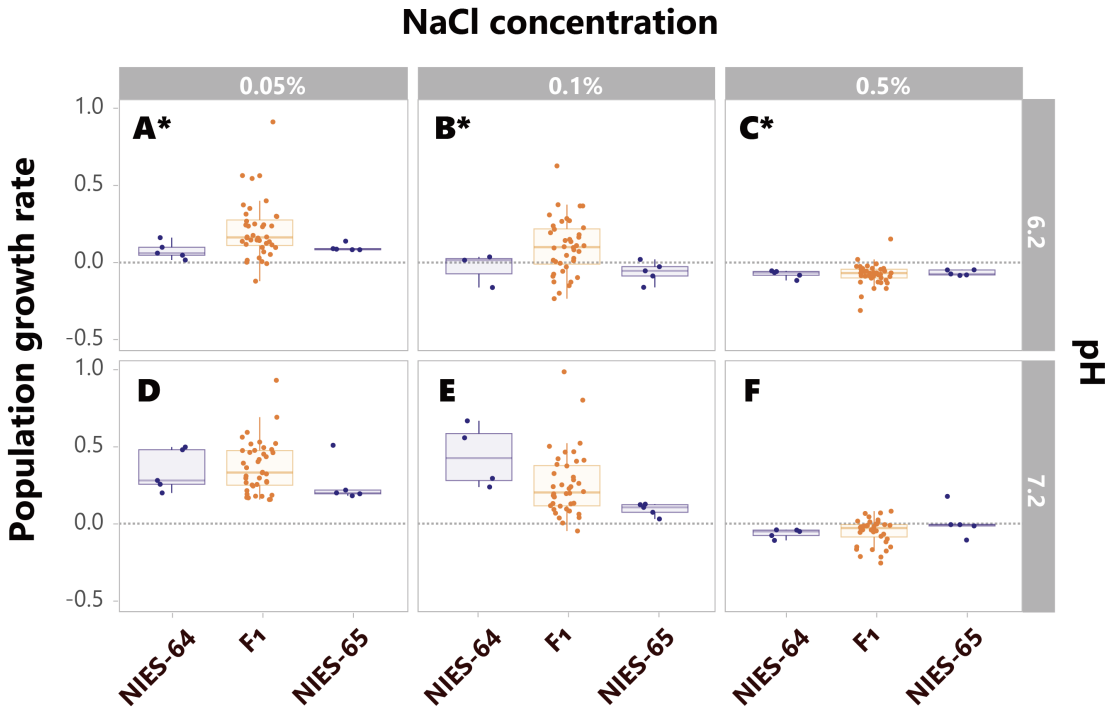


Fig. 2. Population growth rates under each stress condition.

Blue and orange points (and boxplots) denote parental (NIES-64 and NIES-65) and F_1 populations, respectively. Bars and boxes represent the median and interquartile range, respectively. Whiskers extend to 1.5 times the interquartile range. A, D: 0.05% NaCl. B, E: 0.1% NaCl. C, F: 0.5% NaCl. A-C: pH 6.2. D-F: pH 7.2. Under condition marked with an asterisk (A, B, C), the F_1 populations showed significantly larger variation in growth rates than the parental ones ($p < 0.01$). See Fig. S3 and S4 for more details and Fig. S5 for the effects of the founder population size.