Higher rates of sex evolve during adaptation to more complex environments

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Edited by Sarah P. Otto, University of British Columbia, Vancouver, BC, Canada, and approved November 28, 2016 (received for review March 10, 2016)

A leading hypothesis for the evolutionary maintenance of sexual reproduction proposes that sex is advantageous because it facilitates adaptation. Changes in the environment stimulate adaptation but not all changes are equivalent; a change may occur along one or multiple environmental dimensions. In two evolution experiments with the facultatively sexual rotifer *Brachionus calyciflorus*, we test how environmental complexity affects the evolution of sex by adapting replicate populations to various environments that differ from the original along one, two, or three environmental dimensions. Three different estimates of fitness (growth, lifetime reproduction, and population density) confirmed that populations adapted to their new environment. Growth measures revealed an intriguing cost of complex adaptations: populations that adapted to more complex environments lost greater amounts of fitness in the original environment. Furthermore, both experiments showed that *B. calyciflorus* became more sexual when adapting to a greater number of environmental dimensions. Common garden experiments confirmed that observed changes in sex were heritable. As environments in nature are inherently complex these findings help explain why sex is maintained in natural populations.

evolution of sex | adaptation | Fisher–Muller hypothesis | Hill–Robertson interference

The widespread occurrence of sexual reproduction, despite its well-known costs (1), has puzzled biologists for decades (2–4). Numerous hypotheses have been proposed to explain how the benefits of sex may outweigh its costs (see refs. 3 and 5 for reviews) and, although studies have provided tests of core assumptions (6, 7) or found evidence consistent with theory (8–10), direct experimental evidence of sex evolving within populations remains rare (but see refs. 11 and 12). One popular idea is that sex evolves because it facilitates adaptation. This idea was first proposed by Weismann who suggested that sex generates genetic variation on which selection can act (13). Subsequent modeling work formally demonstrated that sex can be favorable in populations under directional selection. Theory shows that shuffling of genetic material during reproduction can bring together beneficial mutations from different genetic backgrounds (14, 15). Furthermore, it can break down negative linkage that builds up in finite populations under selection (16), indirectly favoring more genetic mixing (17, 18). Experimental studies have demonstrated that populations with sex adapt faster than asexual ones (19–22). Furthermore, experimental evolution studies focusing on the within-population evolution of sex (12), as well as of recombination (23) and selfing (24), support the theory that genetic mixing is favorable during adaptation.

Although both theory and experimental data find evidence for sex being favorable during adaptation, not all types of adaptation are the same. Sometimes organisms are faced with simple environmental challenges and other times environmental challenges are more complex (here defined as a change along multiple environmental dimensions). How does the complexity of the environmental challenge influence the extent to which sex is favored? Complex environmental challenges may impose selection on a greater number of loci than simple environmental changes. If so, then we expect higher levels of sex to evolve in complex environments compared with simple environments because theory predicts stronger selection for sex in finite populations when more loci are under selection (25). Higher levels of sex are also expected to evolve if complex environmental changes generate stronger selection per locus, even if the number of selective targets is unaltered (25). Alternatively, if complex challenges select on different sets of loci rather than altering the number or strength of selective targets, we would expect no relationship between the complexity of the environmental challenge and the evolutionary response of sex.

To understand whether environmental complexity plays a role in the evolution of sex, we performed two evolution experiments using the facultatively sexual rotifer *Brachionus calyciflorus* (Fig. S1 shows lifecycle). We manipulated complexity along one, two, or three environmental dimensions by combining different abiotic stressors in a factorial design (Fig. S2). In the first experiment (exp1), we adapted populations to either increased salinity ([NaCl]), or decreased temperature, or both. In the second experiment (exp2), populations were adapted to increased [NaCl], decreased temperature, and a heavy metal ([$CuSO_4$]), as well as all subsets thereof to create a total of eight different environments differing from the initial conditions along zero (control environment), one, two, or three environmental dimensions. The strength of the abiotic stressors differed slightly between experiments as did the rotifer populations used to initiate the experiment (Fig. S2 shows details). Here we focus on the results of experiment 2 although experiment 1 showed similar patterns (Figs. S3 and S4).

Significance

The existence of sexual reproduction despite its well-known costs is a decades-old puzzle. Although recent studies show that sex can be advantageous because it may facilitate adaptation, it remains unclear whether all types of adaptation result in the same sexual response. Using experimental evolution we show that adaptation to different environments results in varying amounts of sex and, more importantly, that higher levels of sex evolve when adapting to more complex environments. As environments in nature are inherently complex, our findings help explain why so many natural populations maintain such high levels of sex.

Author contributions: P.L., E.K.H.H., and A.F.A. designed research; P.L., E.K.H.H., M.G., S.C., A.S., C.Y., and Y.S.K. performed research; P.L. and A.F.A. analyzed data; and P.L., E.K.H.H., and A.F.A. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1604072114/-/DCSupplemental.

Results and Discussion

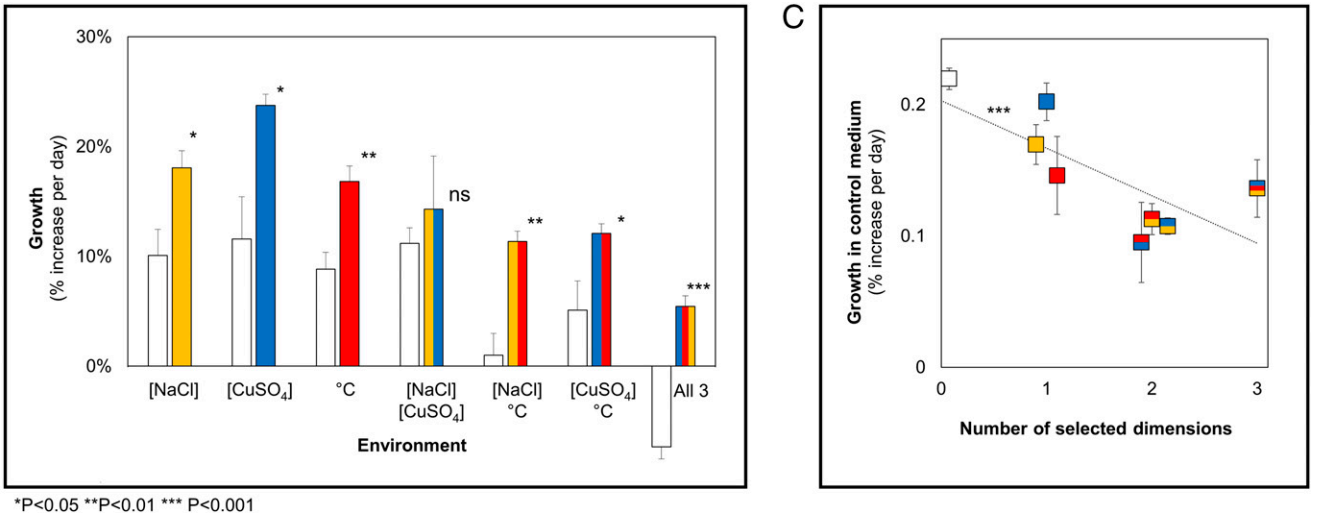
Adaptation. To test whether our populations adapted to their new environments, we use two types of fitness assays (growth assays and lifetime reproduction assays). Before these assays, rotifers were raised in the test environment for at least three (clonal) generations to minimize maternal effects. In growth assays, we estimated population growth rate by allowing an individual rotifer to grow with ad libitum food supply over 5 d, while population densities were recorded. These assays where conducted at the end of experiment 2 for each population in each of the eight environments (with 24 replicates per population per environment, Fig. 1*A*). There is clear evidence of adaptation; evolved populations had higher growth rates than nonevolved (control) rotifers when assayed in the environment they had been adapting to (Fig. 1*B*, significant for all environments except the combination of [NaCl] and [$CuSO_4$]). Lifetime reproduction assays measuring the total number of offspring produced by individual rotifers provide further evidence of adaptation (lifetime reproduction for each of three clonal offspring of 8–10 individuals measured per population for six time points). In both experiments, lifetime reproductive success increased (up to 100%) over time for adapting populations (Fig. S3). Finally, in situ measures of population density show an ecological signature of adaptation. Following the environmental change, population density initially decreased and then slowly recovered or exceeded control densities (exp1: Fig. S4*A*; and exp2: Fig. 2*A* and Fig. S5).

Increased performance in the new environment came at a cost to performance in the original environment. Growth in the original environment (control) was strongly reduced (up to 50%) and the loss of fitness increased with the number of environmental dimensions on which the population was selected (exp2: Fig. 1*C*, $P < 0.001$). This finding suggests a trade-off that increases in strength with increasing environmental complexity. Although trade-offs are well-studied (26, 27), our study shows that larger trade-offs evolve in response to more complex environmental challenges. As natural populations are likely to experience more complex environments than used in typical laboratory selection experiments, our results suggest that trade-offs may be stronger and more common in nature than suggested by laboratory studies. Although our data show a realized trade-off at the phenotypic

A

Evolved line \ Test environment	control	[NaCl]	[$CuSO_4$]	°C	[NaCl] [$CuSO_4$]	[NaCl] °C	[$CuSO_4$] °C	All 3 stressors
Control	**22 .0**	10.1	11.6	8.8	11.2	1.0	5.1	-7.4
[NaCl]	17.0	**18.1**	10.6	9.5	9.7	3.6	6.6	-3.3
[$CuSO_4$]	20.2	10.7	**23.8**	8.2	5.6	6.9	9.0	-1.4
°C	14.6	12.0	11.0	**16.8**	5.9	3.8	8.3	-4.6
[NaCl] & [$CuSO_4$]	10.7	10.0	12.6	6.5	**14.3**	0.3	6.4	-2.9
[NaCl] & °C	11.3	9.3	15.6	10.8	8.2	**11.4**	9.2	1.6
[$CuSO_4$] & °C	9.5	6.1	13.0	9.4	4.7	1.6	**12.1**	-2.3
All 3 stressors	13.6	11.6	11.1	9.2	12.3	2.7	7.2	**5.4**

100% Growth 0%



*P<0.05 **P<0.01 *** P<0.001

Fig. 1. Adaptation. (*A*) Growth for each combination of evolved line and environment was estimated at the end of experiment 2 by counting the number of rotifers in a population initiated by a single individual for 5 d and fitting a slope to these observations. Within each column, cells are shaded to reflect growth rate relative to the maximum growth rate observed in that environment (i.e., the darkest cell within each column represents the maximum growth rate among the eight treatments tested in that environment; lighter shading indicates reduced relative growth rate; no or negative growth is shown in white). Results show that adaptation occurred, evolved lines performed better in the new environment than nonevolved rotifers (the diagonal element has the highest value within each column). (*B*) Comparison of the growth of adapted lines in their own environment to the controls in that environment (graphical representation of *A*, first row vs. diagonal). (*C*) Adaptation to increasing complex environment leads to a greater loss of fitness in the ancestral environment (graphical representation of *A*, first column). For estimates of fitness over the course of both experiments, see Fig. S3. Error bars represent 1 SE. ns, not significant. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

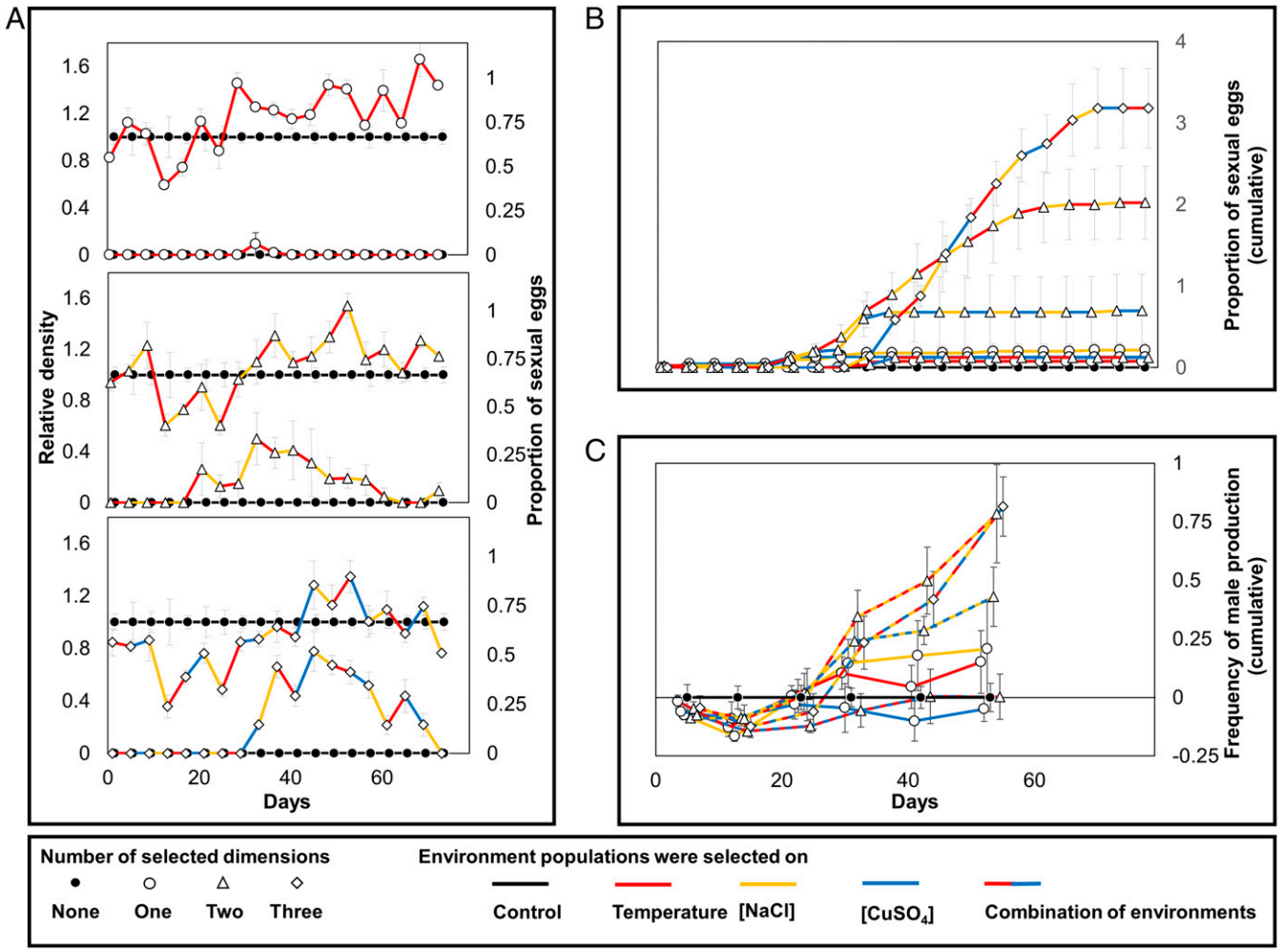


Fig. 2. Evolution of sex. Measures of sex for experiment 2 (Fig. S4 shows similar measures for experiment 1). The relative density (*Upper* lines on primary axis) and proportion of sexual eggs (*Lower* lines on secondary axis), in populations adapting to temperature, [NaCl] and temperature, and all three stressors (*A*, other environments in Fig. S5). Populations adapting to more complex environments produce relatively more sexual than asexual eggs (*B*, represents the area under the proportion of sexual eggs curves depicted in *A* and Fig. S5). These changes in sex were heritable; autoinduction assays that measured the production of males in a common environment detected an increase in the frequency of genotypes that produced males during the same time period as in situ population measures (compare *B* and *C*, see correlation in Fig. S7). (*C*) The frequency is given as the difference between the frequency observed in a focal treatment minus the frequency in the nonadapting control (i.e., values can be negative).

level (increased fitness in the new environment is associated with reduced fitness in the original environment), such data do not necessarily indicate gene-level trade-offs (i.e., antagonistic pleiotropy). Indeed, differences in the strength, but not direction, of selection on different loci between environments (e.g., conditional neutrality), combined with negative disequilibria, can yield similar patterns (28, 29). Regardless of whether antagonistic pleiotropy or disequilibria are responsible, the results show an intriguing realized cost to complex adaptations (which differs from the "cost of complexity" discussed in refs. 14, 30, among others).

Growth measurements also suggest that the three abiotic stressors we used are uncorrelated in their fitness effects. Populations adapted to each single stressor show an increase in growth in their own environment compared with the control, but little change in any of the other single stressors (Fig. 1*A*, *Top Left*). Moreover, each population (including those selected along two and three environmental dimensions) does better in its own environment than any other environment (i.e., each diagonal element of Fig. 1*A* has the highest growth rate within its column). These patterns would not be expected if, for example, [NaCl] and [$CuSO_4$], selected for the same alleles. Rather, a likely explanation for these patterns is that different loci underlie adaptation to the three stressors (although it is possible that the targets of selection are partially overlapping). This explanation is consistent with the observation that adaptation to a less complex environment may provide a partial fitness increase in a more complex environment (e.g., *Top* four rows of the last column of Fig. 1*A*). A reasonable interpretation of these patterns is that by increasing environmental complexity, we are likely increasing the number of loci under selection, which according to theory should lead to selection for higher levels of sex (25). However, it is possible that only subsets of the alleles that are responsible for adaptation to each of the single stressors are favored when facing multiple stressors (due to pleiotropic constraints). Without a detailed study of the genetic architecture of adaptation, we cannot know the true number and strength of selective targets in each environment.

Evolution of Sex. Although sex can be favored during adaptation, not all types of adaptation are expected to be equal in this regard. The extent to which sex is expected to evolve during adaptation depends on various aspects of the genetic architecture underlying adaptation, including gene number, effect sizes, dominance and epistasis, and physical and statistical linkage of selected sites to

each other as well as to modifiers of sexual propensity (23, 25, 31). Thus, we should expect differences in the level of sex that evolves in response to adaptation to our three abiotic stressors. Indeed, observations of the proportion of sexually produced eggs (five replicates per population, measured every 4 d) show that there is considerable variation among alternate single stressors. For example, in both experiments 1 and 2, there was a considerable increase in sex in populations adapting to higher [NaCl] but not in populations adapting to low temperature (Fig. 3, orange points representing [NaCl], red points representing temperature) despite the fact that there were dramatic adaptive responses to both stressors (Fig. 1*B*, compare red and orange bars to their respective controls).

Variability in amounts of sex in response to adaptation to our three stressors indicates that sex in more complex environments should be interpreted in relation to the sexual response exhibited in the separate components comprising these environments. For example, although we observe low levels of sex in the populations adapted to the combination of [$CuSO_4$] and low temperature, the sexual response was higher than in populations that adapted to [$CuSO_4$] only or low temperature only. This pattern holds across all treatment combinations; in both experiments, more complex environments always had more sex than the separate stressors they were composed of (Fig. 3, colored points). On average (Fig. 3, black bars), populations adapting along two environmental dimensions showed a greater increase in sex than those adapting along a single dimension (post hoc contrasts using generalized linear hypothesis tests "glht," on one vs. two environmental dimensions following a general linear model (GLM), exp1: $P < 0.001$; exp2: $P = 0.0133$) as did the most complex environment (three stressors) compared with populations adapting to two environments (glht, two vs. three dimensions, exp2: $P < 0.001$).

Further evidence that rates of sex were higher during adaptation to more complex environments comes from "sexual autoinduction" assays, our second measure of sex. For these assays, individual rotifers (up to 60 replicates) were sampled from their respective populations at regular intervals and subsequently grown in a standardized environment (control environment). After three clonal generations in a standardized environment, neonates were left to grow until males were observed, at which point population densities were recorded. This autoinduction assay (32, 33) reflects sexual propensity, as initiation of sexual reproduction in monogonant rotifers depends on a sex-inducing protein they excrete themselves (34). Genotypes that are more prone to sexual reproduction (e.g., responsive to the signal) will go sexual (and produce males) at lower population densities. These assays are inherently noisier but, in general, show similar patterns to those described above. We observed that sexual propensity either decreased (exp1; Fig. S4*C*) or remained constant in the control (exp2; Fig. 2*C*), whereas adapting populations tend to show increasing amounts of sex in more complex environments (significant for the comparison between the most complex environment and the control, ghlt exp2: $P = 0.022$).

In all of our treatments, rates of sex increase during the period of adaptation, then decline. This is the same pattern observed by Becks and Agrawal (11). They showed that genotypes naturally produced by sex had higher average fitness than those naturally produced via asex during adaptation. After populations became reasonably well adapted, genotypes produced by sex had lower average fitness than those produced via asex. The decline in sex is expected. Once adapted, recombination will break up coadapted gene complexes, making sex detrimental. This, in addition to the infamous twofold cost of sex (1) and a longer sexual lifecycle, provides strong selection against sex.

We believe that the observed changes in sex are heritable and not plastic but we do not have definitive evidence that they are genetic. Other studies have reported that sex in rotifers can be dependent on the environment (35). In some, although not all, of our treatments (e.g., those involving [NaCl] in exp1), there is a small increase in our in situ measure of sex immediately following the environmental change (Fig. S4*A*), which may be due to plasticity. However, in most of our treatments, sex does not increase when rotifers were initially exposed to the new environment. Rather, there is a long delay (exp1: 16.3 ± 9.3 d and exp2: 22.4 ± 6.0 d) between the change in the environment and the first observation of sexual eggs and sex continues to increase to up to 7 wk after the environment changed (see the lower of the two colored lines, exp1: Fig. S4*A*; exp2: Fig. 2*A* and Fig. S5). Although the sexual cycle takes longer to complete (~6 d) than the asexual cycle (~2 d), the observed period is much longer than expected if the changes were due to a direct plastic response. In addition to simple plastic responses, in some systems, exposure to an environmental cue must occur over multiple generations to trigger a plastic response (36). We tested for this possibility by propagating rotifers for 40 d as clonal lines in each of the eight environments used in exp2 (i.e., approximately the time period over which we observed evolutionary responses in exp2). In this test, a plastic response to the environmental change is possible but not a genetic response because there is no genetic variation within clonal lines. At the end of this experiment we performed sexual autoinduction assays in the control environment for all lines (as in exp2). We did not observe an increase in sex for lines maintained in any of the stressful environments relative to those maintained for the 40 d in the control environment (Fig. S6). This result indicates that multigenerational exposure to these stressors does not elicit a plastic sexual response that can explain the elevated rates of sex we observed in our experimental populations.

Our main argument against plasticity as the sole explanation for our results is that the observed changes in sex were heritable; differences between control and evolved populations were present when sex was assessed with autoinduction assays after three clonal generations in a standardized (common) environment (Fig. 2*C*). These measures were correlated with in situ observations of the proportion of sexual eggs ($R^2 = 0.61$, $P < 0.001$, Fig. S7) and changes in both measures happen on the same time scale (compare Fig. 2 *B* and *C*). Although we observe a heritable response, we do not know whether the underlying mechanism of heritability is genetic or epigenetic. Regardless, the theory for the evolution of sex applies if the "high-sex" allele differs from the "low-sex" allele due to a DNA sequence difference or due to an epigenetic modification. Of course, the long-term persistence of the evolved

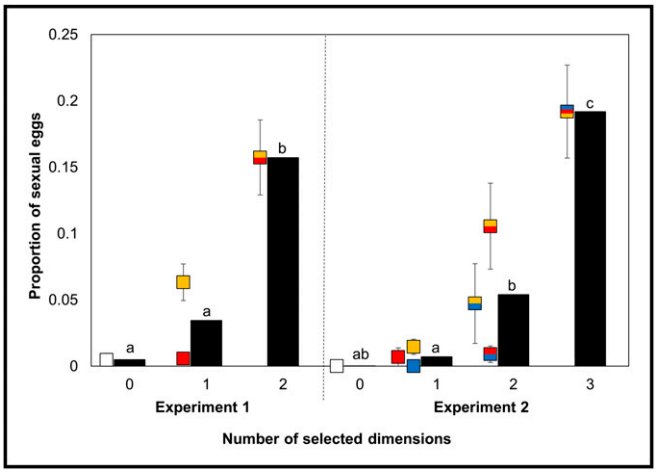


Fig. 3. Average proportion of sexually produced eggs. Average proportion of sexually produced eggs over all time points in both experiments (±SE). Individual treatments depicted by colored points and the averages across treatments are depicted by black bars. Letters represent significant differences at the $P = 0.05$ level based on custom contrasts (glht) after GLM.

effect depends on the stability of difference between these alleles. Although we argue that heritable changes contribute to our observed patterns, we expect that plasticity also has a role. As adaptation proceeds, density increases. Any heritable change in sexual propensity that evolves as a byproduct of adaptation will thus be exaggerated by exposure to the increasing density, magnifying the in situ response relative to what was observed in common garden autoinduction assays.

In *B. calyciflorus* sexually produced eggs are also resting stages. The in situ increase of sex with greater environmental complexity could thus in part be caused by an accumulation of unhatched sexual eggs if hatching rates are lower in more stressful environments. However, this was not the case; hatching success of rotifers from this source population is equal among all our treatments (Fig. S8). Our results could also be confounded if there was selection for resting stages rather than sex. However, this seems unlikely as increased production of resting stages in more stressful environments provides no evolutionary advantage if the stressor persists (i.e., because it is impossible to "wait out" the stress via production of resting eggs, there would be no direct selection for production of such eggs). Furthermore, our experimental design may actually put resting eggs at a (slight) disadvantage. Populations are maintained under semicontinuous growth by replacing 10% of the culture every 2 d. Although resting eggs from our source populations hatch spontaneously after a few days (4 d, Fig. S8), asexual eggs are able to leave proportionally more offspring under these conditions as they hatch and start reproduction earlier. This ongoing selection against resting stages may contribute to the observed rapid decline of sex as adaptation slows.

Although a within-population advantage for sex during adaptation to new environments has been shown previously (12), our findings demonstrate that adaptation to different environments results in varying amounts of sex and, more importantly, that higher levels of sex evolve in response to adaptation along more environmental dimensions. Theory predicts that higher levels of sex should evolve with more or stronger targets of selection (25). Whereas the genetic basis of adaptation here is not known, an interpretation of our results is that more complex environments provide more or stronger targets of selection, thereby generating stronger selection for sex. Given that populations in nature are inherently complex, likely with many loci under selection at any time, our findings may help explain why natural populations maintain such high levels of sex. Although sex was not maintained indefinitely in our experiments and rapidly decreased after populations reached their fitness maxima, the loss of sex is unlikely in nature as selection will seldom be constant (37, 38). Indeed, the finding of a fitness trade-off that increases in strength with environmental complexity supports the notion that sex is favorable in complex and variable environments (39, 40). If adaptation to one environment results in the loss of fitness in another, there will be an advantage for sex if the environment reverts to its original state.

Materials and Methods

Experimental Overview. In exp1, rotifers were adapted to increased salinity [NaCl] and lower temperature or both. In exp2, rotifers were adapted to combinations of [NaCl], lower temperature, and [$CuSO_4$]. Experimental populations (four populations per environment) consisting of ~6,000 rotifers were initiated from laboratory stocks at least 1 mo before induction of environmental changes, and experiments lasted for ~70 d (30–40 generations). During experiments, the number of females, asexual eggs and sexual eggs, were enumerated every 4 d in each of the populations. To verify that adaptation occurred, we sampled individual rotifers from each population at six time points during the experiment and estimated their lifetime reproductive success. Furthermore, at the end of exp2, we estimated growth rates of each of the evolved and nonevolved populations in every environment (64 combinations). Finally, to ensure that in situ measurements of sexual eggs reflected evolutionary changes, we also measured sex of all populations in a common environment at six or more time points during the experiment.

Experimental Setup and Maintenance. The source of the rotifers used in both experiments was Lake Onondaga, New York. Two separate rotifer stocks were used: rotifer stock S2 (exp1) was hatched in November 2012 from sediment collected in spring 2009, and rotifer stock S3 (exp2) was hatched in October 2013 from sediment provided by N. G. Hairston Jr. Stock populations were kept in Erlenmeyer flasks with artificial medium (Table S1) under 24-h light at 22 °C. Populations were maintained as semicontinuous cultures by replacing 10% of the medium every other day with fresh medium containing chemostat cultured algae (*Monoraphidium minutum*, SAG 278–3, Algae Collection, University of Goettingen). Rotifers were kept under these conditions for at least 6 mo before the start of experiments. Experimental populations were created from multiple flasks of these stock populations (well mixed) and kept in 500-mL tissue culture flasks (Sarstedt). Populations were maintained by replacing 10% (50 mL) of medium every other day and feeding 100 million algae daily. After a period of 1 mo, the experiments were initiated and the environment was gradually changed to the predetermined treatments over an 8-d period. Temperature was decreased from 22 °C to 18.5 °C or 17.5 °C (for exp1 and exp2, respectively) by incrementally lowering the temperature setting of the incubator every 2 d (starting after day 1 for exp1 and after day 4 for exp2). Similarly, increasing amounts of table salt [NaCl] were added to reach final concentrations of 0.32 g/L (exp1) or 0.4 g/L (exp2) by day 8. Finally, 1.25 μg of $CuSO_4$ (exp2) was added to appropriate populations daily. As copper binds to biological material, the final concentration of this stressor is unknown but as evident in the results presented here (Fig. S5), it did reach its intended effect over the 8-d period. As population size is known to influence the evolution of sex (37), we attempted to reduce variation in size by standardizing population densities (including control populations) to the fourth lowest population across all populations (informed by density counts taken earlier on the same day) every fourth day while populations were adapting to their new environment (Fig. S9). This was done by filtering the appropriate amount of medium over a 60-μm filter and discarding any rotifer caught while replacing the medium.

Estimates of Fitness. We used three different estimators of fitness: lifetime reproductive success (exp1 and exp2), growth rate (exp2) and population density (exp1 and exp2). Before measuring lifetime reproductive success and growth rate, we standardized maternal effects by keeping rotifers sampled from their respective populations in the test environment for at least three clonal generations (each replicate was kept in 1 mL of medium with 500,000 algae and transferred every 3 d). Lifetime reproductive success was estimated by following a single juvenile rotifer until death and recording the number of offspring produced. The focal rotifer was transferred to a new well plate (with 1 mL medium and 500,000 algae) daily to avoid confounding the focal rotifer with its offspring. For each population, we tested three clonal offspring (replicates) of 8–10 individuals (24–30 rotifers total) at six time points during the experiment. In addition, at the end of experiment 2 we performed growth assays where a single rotifer was placed in a well (with 1 mL medium and 1 million algae) and was allowed to propagate for up to 5 d. Each day the number of rotifers was recorded and growth was obtained by fitting a linear regression to the log transformed data [ln (data + 1)] for each of the 24 replicates. These assays were performed for each population in all selective environments. As an ecological signature of adaptation, we measured population density every 4 d by taking five 1-mL samples of each population and counting the number of females (and males).

Sexual Propensity. We measured rates of sex within each population by estimating the proportion of eggs produced sexually ("proportion of sexual eggs"). Sexual and asexual eggs were counted simultaneously with population density following the same protocol. In addition, we performed sexual autoinduction assays in a common garden (control environment) to confirm that changes in the in situ proportion of sexual eggs were not caused by a plastic response to the environmental change. Before the assays, we removed maternal effects as described above so that tests were performed using individuals that had been maintained in the common garden conditions for at least three clonal generations before the assay. Each assay replicate was initiated by placing a single juvenile rotifer in a well (12-well plate for exp1, 24-well plate for exp2), which was fed ad libitum, and supplied with additional vitamins until it produced a male. Wells were checked every day for the occurrence of males and density at first male was recorded. If no males were recorded after 13 d, the density was estimated and the assay was terminated.

Statistical Analysis. Statistical analyses were performed with *R* (41). Two populations went extinct during experiment 2 and were excluded from all analyses (one [$CuSO_4$] population and one [$CuSO_4$] and [NaCl] population).

Growth data on adaptation was analyzed using *t* tests comparing the evolved populations to the control within each environment (Fig. 1*B*). The loss of growth rate in the original environment was examined using a linear model with number of selected dimensions as the explanatory variable (Fig. 1*C*). Both measures of sex, the proportion of sexual eggs and sexual autoinduction, were analyzed using a GLM with environment as the explanatory variable (proportion of sexual eggs in exp1 and sexual autoinduction in exp2 were analyzed with quasibinomial error structure and proportion of sexual eggs in exp2 with binomial error structure; experiment 1 autoinduction assays were not analyzed due to problems with control measurements, Fig. S4*C*). Generalized linear hypothesis tests (glht, package multicomp) were performed following GLM to determine the effect of the number of environmental dimensions rotifers were selected on (i.e., post hoc custom contrasts "sex in control environment − sex in populations selected along one environmental dimension = 0," "sex in one dimension − sex in two dimensions = 0," etc.). Analyses for proportion of sexual eggs were performed on the cumulative number of asexual and sexual eggs produced over the whole experiment since the environmental change. A small number (0.5) was added to each of the control and copper populations of experiment 2 to allow for model estimates as no sexual eggs were observed in these populations. Values of zero cause computational problems with the statistical model and adding a small number to such cases is one recommended way to deal with this issue (42). Adding a small number to these populations is conservative as it decreases the observed difference between the environments. For autoinduction assays, we determined the density at which the first male was produced. However, as some replicates never produced males, even after reaching high densities (>200 rotifers per milliliter), the data were treated as a binary variable. Rotifers that made a male under a threshold density (36 rotifers per milliliter) were considered sexual, whereas those that did not were considered asexual (and thus were analyzed as not having produced a male). By applying the threshold to each of our replicates (up to 60 replicates per population per time point) we calculated the frequency of replicates that produced males at the threshold density. Alternative thresholds (26 or 46 rotifers per milliliter) did not change the patterns in the data (alternative thresholds were highly correlated with the used threshold of 36 rotifers per milliliter; $r = 0.86$, $P < 0.001$ for 26 rotifers per milliliter and $r = 0.95$, $P < 0.001$ for 46 rotifers per milliliter). GLM analysis for autoinduction assays was performed on the total number of rotifers considered sexual or asexual over the whole experiment since the environmental change.

ACKNOWLEDGMENTS. We thank Nelson Hairston Jr. for providing sediment samples with rotifer eggs, Asher Cutter for use of laboratory equipment, and Yuheng Huang for providing critical comments on our manuscript. This work was supported by National Sciences and Engineering Research Council of Canada Discovery Grant 2015-05387 and Swiss National Science Foundation Fellowship PBBSP3-138671.

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