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To cite this version:

Elsa Noël, Philippe Jarne, Sylvain Glémin, Alicia Mackenzie, Adeline Segard, et al.. Experimental Evidence for the Negative Effects of Self-Fertilization on the Adaptive Potential of Populations. Current Biology - CB, 2017, 27 (2), pp.237-242. 10.1016/j.cub.2016.11.015 . hal-01456775

HAL Id: hal-01456775
https://hal.science/hal-01456775

Submitted on 7 Feb 2017

Experimental Evidence for the Negative Effects of Self-Fertilization on the Adaptive Potential of Populations

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SUMMARY

Self-fertilization is widely believed to be an "evolu-tionary dead end" [1, 2], increasing the risk of extinction [3] and the accumulation of deleterious mutations in genomes [4]. Strikingly, while the failure to adapt has always been central to the dead-end hypothesis [1, 2], there are no quantita-tive genetic selection experiments comparing the response to positive selection in selfing versus outcrossing populations. Here we studied the response to selection on a morphological trait in laboratory populations of a hermaphroditic, self-fertile snail under either selfing or outcrossing. We applied both treatments to two types of populations: some having undergone frequent selfing and purged a substantial fraction of their mutation load in their recent history [5], and others continu-ously maintained under outcrossing. Populations with a history of outcrossing respond faster to selection than those that have experienced selfing. In addition, when self-fertilization occurs during selection, the response is initially fast but then rapidly slows, while outcrossing populations main-tain their response throughout the experiment. This occurs irrespective of past selfing history, suggesting that high levels of inbreeding depression, contrary to expectation [6], do not set strong limits to the response to selection under inbreeding, at least at the timescale of a few generations. More surprisingly, phenotypic variance is consistently higher under selfing, although it quickly becomes less responsive to selection. This implies an in-crease in non-heritable variance, hence a break-down of developmental canalization [7] under selfing. Our findings provide the first empirical support of the short-term positive and long-term negative effects of selfing on adaptive potential.

RESULTS AND DISCUSSION

Self-fertilization has several short-term advantages, including a higher fidelity of gene transmission across generations, a more efficient exposition of alleles to selection, and reproductive assurance when pollinators or partners are scarce [8, 9]. However, it is also expected to decrease adaptive potential in the long term by increasing genetic drift and limiting recombina-tion efficiency at the genome scale: selfing populations should display lower quantitative genetic variance than outcrossing populations of similar census size [10] and have a reduced ability both to respond to positive selection from standing variation [11] and to fix several advantageous alleles at once (selective interference [12]). The impact of selfing on adaptive responses to environmental change was thus initially the core of the "evolutionary dead end" argument.

We addressed this central question in the hermaphroditic outcrossing snail *Physa acuta* via the following questions: (1) Do populations regularly exposed to self-fertilization lose additive genetic variance (adaptive potential) compared to outcrossing ones? (2) Does self-fertilization during selection affect the popu-lation response to selection? (3) Does inbreeding depression strongly limit the ability to respond to selection under self-fertilization? We constructed two types of experimental evolution lines (each line being a laboratory population of N z 80 adults; two replicates per type): individuals from C (outcrossing) lines al-ways outcrossed, whereas self-fertilization was imposed every other generation in S (frequent selfing) lines [5]. Each type was represented by two independent replicates. After around 30 gen-erations, we performed two parallel selection experiments within each line, one under 100% selfing and the other under 100% outcrossing. In both cases, we measured 200 adults each gener-ation and retained the 50 (1/4; Figure 1) with the highest shell width-to-length ratio (hereafter "shell roundness," a trait known to be under selection in natural contexts; see Supplemental Experimental Procedures). In order to specify our expectations, we used individual-based simulations of experimental evolution followed by selection on a multilocus trait, mimicking our experimental conditions, accounting for deleterious mutations and inbreeding depression (see Supplemental Experimental Procedures).

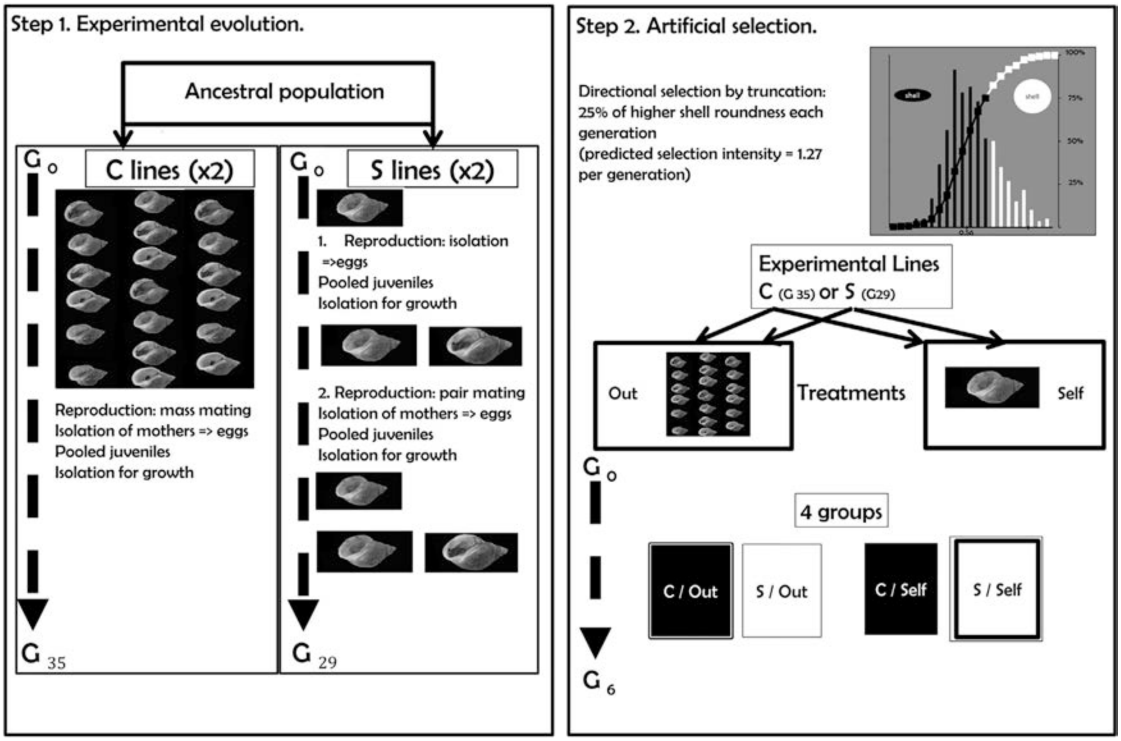

Figure 1. Experimental Protocol

Step 1: experimental evolution of the two lines C and S. Step 2: artificial selection experiment leading to the four groups (each including two replicates). See also Figure S1.

Self-Fertilization Enhances the Response to Selection at First but Compromises It Later

In all eight populations (two types 3 two replicates 3 two mating treatments during six generations of selection; 12,000 indi-viduals measured), we observed significant increases in shell roundness over generations ($p < 0.001$; Table 1; Figure 2A3), while unselected experimental evolution lines (1,200 individuals measured) underwent no significant changes during the same period (Table S1; Figure S1). Both C and S populations re-sponded to selection under outcrossing (Figures 2A2 and 2A3), but the phenotypic change per generation, which reflects the ad-ditive genetic variance, was higher in C than in S populations in both the first and second halves of the experiment (“line type 3 generation”: $p = 0.015$ and $p < 0.001$, respectively; the dataset was broken in two halves, as the response was not linear over the entire period). Thus, 30 generations of frequent selfing substantially decreased the additive variance for quantitative traits, providing a positive answer to our first question. As no selection was applied on shell roundness over these 30 generations, this effect likely resulted from an acceleration of genetic drift. Gene diversity at microsatellite markers (7 microsatellites, 654 geno-typed individuals) was reduced in S relative to C lines (⍰27%, 0.271 versus 0.376; Figure S2) and was not significantly different from the loss in quantitative variance, since the corresponding reduction in response to selection (realized heritability, 0.19 versus 0.33, ⍰ 42%) does not lie outside the estimation error around the neutral estimate ($p = 0.51$, two-sided test based on 1,000 bootstraps over microsatellite loci). Theory predicts that selfing accelerates genetic drift by increasing homozygosity [13] and by reducing effective recombination, which allows link-age disequilibria to accumulate, causing selection at any locus to lower genetic diversity at linked loci [14]. Both effects are ex-pected to remain moderate under the alternate outcrossing/self-ing regime of S lines, as heterozygosity never drops by more than 50% and linkage disequilibria do not accumulate significantly either (Figure S3). Accordingly, our individual-based simulations predicted only a small difference in realized heritability between S and C lines (on average ⍰ 11%; Figure 2B1). The observed difference (Figure 2B) is significantly larger (⍰42%, a value reached with probability $p = 0.004$ in 1,000 simulations), sug-gesting that selfing reduces effective size through additional processes not represented in the simulations. A possible expla-nation is maternal inbreeding effects whereby some inbred females lay low-quality eggs, potentially leading to the loss of entire families, hence reducing N_e. In the future, it would be inter-esting to investigate how the reduction of N_e under selfing is impacted by different genetic architectures of the mutation load (e.g., epistasis).

We addressed the second question by comparing the selfing and outcrossing treatments. Self-fertilization, when coupled with selection, should initially accelerate the response to selec-tion because it increases the genetic parent-offspring resem-blance, including the additive effects on traits, compared to random mating [15]. Selfing treatment may also bear identical allelic combinations at one or several loci, so that dominance and epistatic interactions become heritable [16]. However this

Table 1. Regression of Individual Shell Roundness on Generations for Lines and Treatments

Effect	χ^2	p
Before Bp		
Generation	51.53	7×10^{-13}
Line × generation	5.95	0.015
Treatment × generation	23.59	1.2×10^{-6}
Line × treatment × generation	2.63	0.104
After Bp		
Generation	70.64	$<2 \times 10^{-16}$
Line × generation	28.99	7.3×10^{-8}
Treatment × generation	5.59	0.018
Line × treatment × generation	1.43	0.231

The individual phenotypic values were regressed on the number of selection generations, and the factors are experimental evolution line (C and S) and treatment (outcrossing and selfing). We considered all interactions. Line, treatment, and their interactions were modeled as fixed effects. Because a breakpoint (Bp) in the response to selection was detected (see Figure 2B2), the generation effect (response slope) was estimated both before and after the Bp. Replicate line within each line (two per line) and temporal periods of one month (21 periods) were modeled as random effects. The values reported are χ^2 and the probability associated with likelihood ratio tests (one degree of freedom in all tests). See also Tables S1 S3.

short-term positive effect of selfing is counteracted by the erosion of genetic diversity due to selective interferences result-ing from reduced effective recombination [10, 12]. In our exper-iment, we observed both effects (Table 1; Figure 2). Selfing populations responded more quickly than outcrossing ones dur-ing the first three generations (“treatment 3 generation before breakpoint” interaction: $p < 0.001$) but more slowly afterward (“treatment 3 generation after breakpoint” interaction: $p = 0.018$), while the response to selection in outcrossing popula-tions did not slow significantly ($p = 0.231$). All of this broadly matched simulation results (compare Figures 2A1, 2A2, and 2A3).

Our third question related to inbreeding depression, thought to be a limit to adaptation in inbred populations [6]. For example, experimental populations of Tribolium that are declining following an environmental change can be rescued by immigra-tion, which relieves inbreeding depression, giving populations enough time to adapt [17]. High inbreeding depression results in low population fitness and demographic risks but also heightens selective interference effects as deleterious alleles are made homozygous and exposed to selection [18]. In our experiment, extinction risks were removed by population regula-tion at each generation but selective interference remained, as selfing populations had to purge deleterious alleles and respond to selection on shell roundness at the same time. In this context, populations with previous experience of self-fertilization, which have already purged a large part of their inbreeding depression, may maintain their response to selection for a longer time. Inbreeding depression on survival was indeed lower in S than in C lines (0.11 ± 0.12 and 0.50 ± 0.11, respectively; see [5]), but both the decrease in selection response under selfing and the difference in per-generation phenotypic change between outcrossing and selfing treatments (non-significant three-way interactions in Table 1) were similar in S and C lines. Our simula-tions did not uncover such a difference either, casting doubt on whether inbreeding depression can detectably alter the adaptive potential under selfing once demographic risks are removed. This deserves further exploration, given that our simulations pre-dicted a smaller loss of quantitative variance in S lines than observed (see above) and may therefore have underestimated selfing impacts. All in all, the total phenotypic progress was lower in S than in C populations after six generations of selection (Fig-ure 2A3), irrespective of the mating system. This shows that the advantage of lower inbreeding depression in S lines did not compensate for the loss of genetic variance that took place during their 30 generations of alternate selfing.

Origin of Differences between Selfing and Outcrossing: More Than Erosion of Genetic Variance

All lines experienced the same selection intensity because a constant fraction of the shell roundness distribution was selected per generation. Differences in the rate of pheno-typic change may therefore arise for two non-exclusive rea-sons: (1) the phenotypic variance may be larger in some lines, resulting in a larger selection differential (i.e., the average difference in roundness between the upper quartile and the whole distribution), and/or (2) the fraction of variance that is genetically transmitted over generations may be larger in some groups (i.e., there may be a higher phenotypic change per unit of selection differential). In order to disentangle these effects, we plotted both the phenotypic values against cumulative selection differentials (CSD) (Figures 2B2 and 2B3) and the CSD against selection generations (Figures 2C2 and 2C3).

Based on our simulations, we expected the differences in response to selection to stem from differences in the phenotypic response per unit of selection differential, rather than from changes in the selection differential itself (Figures 2B1 and 2C1). However, our results revealed unanticipated effects of self-ing. In S lines, the phenotypic change per unit of CSD was initially larger under the selfing than under the outcrossing treatment, and was then lower, as predicted by simulations (treatment 3 CSD interaction, $p < 0.001$ before and after generation 3; Figures 2B2 and 2B3; Table S2). However, the selection differential per generation remained consistently higher under selfing than under outcrossing (regression of CSD over generations, treat-ment 3 generation interaction; $p < 0.001$ both before and after generation 3). There was no detectable change in time, as attested by linear increases in CSD with generation number (Fig-ures 2C2 and 2C3; Table S3). The pattern of change in CSD with generations matched that of phenotypic variance, which was consistently larger in the selfing treatment than under outcross-ing throughout the experiment (regression of phenotypic SD on generations: treatment effect, $p < 0.001$). This effect was not predicted by our simulations. The decrease in the response to selection under selfing, in spite of a higher phenotypic variance and selection differential, implies that the phenotypic variance under selfing includes a component not responsive to selection, remaining high throughout the six generations of selection while the genetic variance is progressively eroded. This is consistent with a slightly higher phenotypic variance in S than in C lines in the unselected controls (standard deviations 0.022 versus

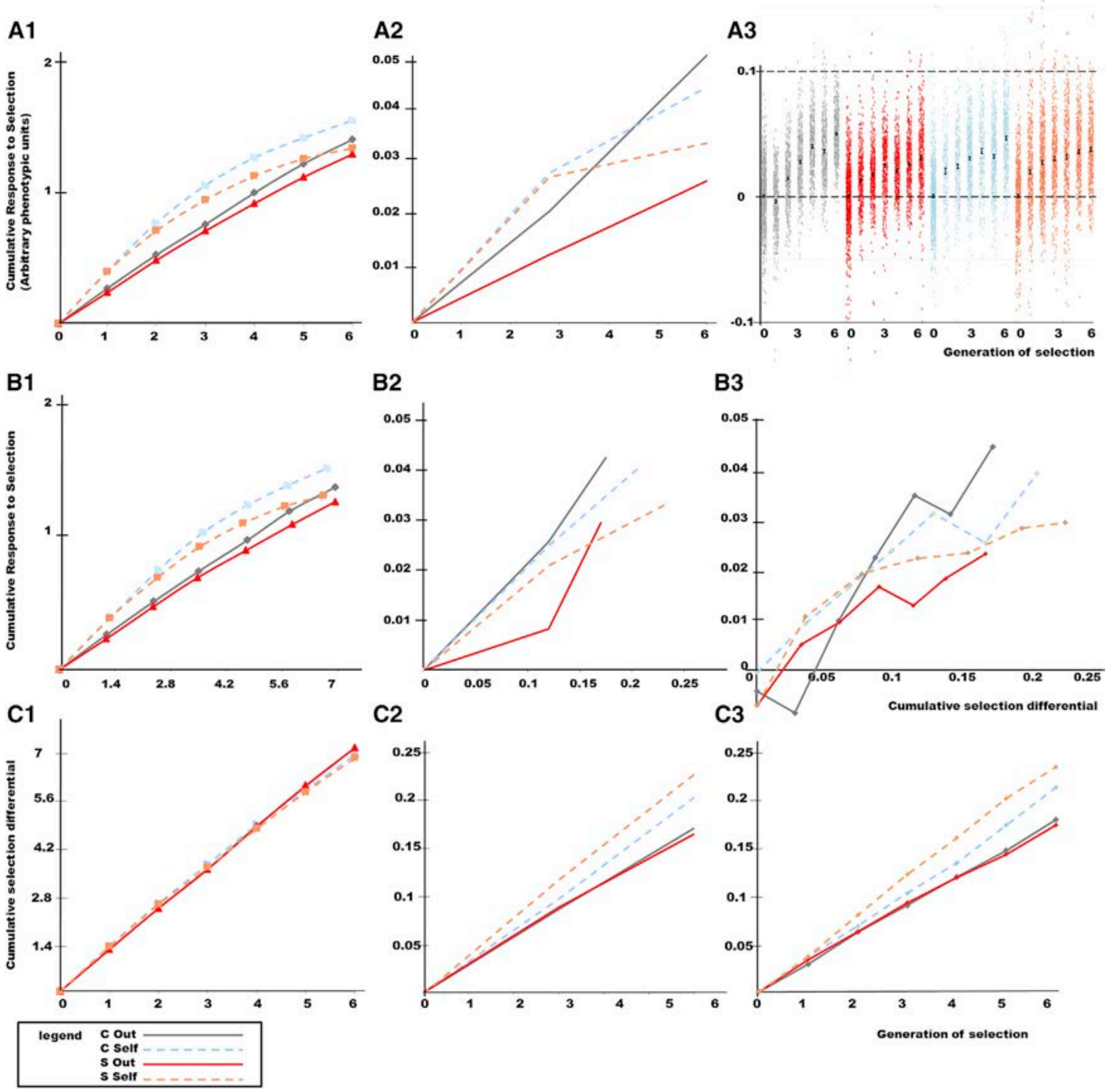

Figure 2. Simulation Results and Results of the Selection Experiments in *Physa acuta*

Simulation results are shown in the left column; experimental results are shown in the middle and right columns. Data legend is beneath (C1). See Tables 1, S2, and S3 for the results of statistical tests associated with (A2), (B2), and (C2) respectively. See also Figures S1 S3.

(A1) Simulations for cumulative response to selection (in arbitrary phenotypic units) as a function of time (in number of selection generation).

(A2) Regression of individual shell roundness on time (number of selection generations) for lines and treatments. Because the response was not linear in all lines, we used a maximum likelihood piecewise regression (details in Supplemental Experimental Procedures). We detected a significant breakpoint at the third generation (2.81; 95% confidence interval: 2.70 3.11), roughly the middle of our experiment. The model estimates regression slopes before and after the breakpoint, while imposing continuity at generation 3.

(A3) Individual and mean ± SEM phenotypic deviations (phenotype minus the estimated mean at generation zero, before selection) per generation in all four conditions, presented side by side to improve readability.

(B1) Simulations for cumulative response to selection as a function of cumulative selection differential (CSD).

(B2) Regression of individual shell roundness on CSD for lines and treatments. A breakpoint is enforced at generation 3 (see Table S2).

(B3) Mean phenotypic deviations (mean phenotype minus estimated mean at generation zero) as a function of CSD.

(C1) Simulations of CSD as a function of time (generations).

(C2) Regression of CSD on time (generations) for lines and treatments. A breakpoint is enforced at generation 3.

(C3) Actual CSD as a function of generations.

0.020, p = 0.03), although C lines respond better to selection. This increase in non-genetic components of variance with inbreeding has been classically observed and interpreted as a decrease in developmental homeostasis or “decanalization” [7, 19]. The reduced response to selection after a few generations of selfing may therefore reflect a combination of fast erosion of genetic variance and increased developmental instability.

Conclusions

Some ideas in biology have become classical with surprisingly little empirical evidence, for example that selfing species are less able to cope with changes in selection regimes than out-crossing ones, as suggested by theoretical models [11, 14]. Although phylogenetic and genomic studies suggest higher extinction rates in selfing plants [3, 20] and a tendency to accu-mulate more deleterious mutations [21], we have little direct empirical evidence on how selfing modifies the adaptive poten-tial at the scale of a few generations to a few tens of generations. Isogenic lines of *Caenorhabditis elegans* survive less well when selfing than when partially outcrossing under artificially elevated mutation and/or environmental changes [22]. However, isogenic lines do not mimic natural populations because they lack initial standing variation, relying on new mutations.

Our study clearly supports the idea that self-fertilization limits adaptive potential. Starting from the same standing genetic vari-ance, populations switching to self-fertilization initially undergo an accelerated response to selection due to homozygosity and the recruitment of dominance and epistatic effects into the her-itable variance. This effect is quickly annihilated by a fast erosion of genetic variance, as selfing populations become progressively unable to generate new genetic combinations through recombi-nation. In addition, our study suggests that even discontinuous selfing is sufficient to decrease the additive genetic variance in a few tens of generations. These patterns are broadly consistent with the observation that wild populations of selfing species are usually less genetically variable than outcrossing ones at both neutral markers [22, 23] and phenotypic traits [18]. In nature, ge-netic erosion will obviously depend on parameters such as pop-ulation size and selfing rate. Our lines are rather small, and selfing rates varied between 50% (periodic selfing before selection started) and 100% (during selection), but we did not place our-selves in extreme low-standing variance conditions. Indeed, the level of genetic diversity of selfing species is usually of the same order as that of our laboratory populations [24]. Moreover, the timescale at which self-fertilization limits the adaptive poten-tial in our experiments is remarkably short: six generations of pure selfing or thirty generations of periodic selfing. It is therefore likely that recurrent selfing reduces the adaptive potential of populations in a large range of naturally occurring selfing rates and population sizes. Of course, this also depends on the ge-netic architecture of traits under selection: selfing in large popu-lations is unlikely to limit the fixation of strongly beneficial alleles arising by mutation but still reduces the standing variance due to small-effect alleles and the possibility of combining several beneficial mutations.

Our study also yielded some unexpected results. First, inbreeding depression did not appear to strongly limit adapta-tion in selfing populations. Populations suddenly exposed to inbreeding through a reduction in size [6, 25] or an increase in the selfing rate [26] expose their recessive load to selection. The resulting genetic deaths may incur demographic risks and decrease the ability to simultaneously respond to other selec-tive pressures [6], for example selection on increased shell roundness. However, previous experience with selfing and the associated partial purge of inbreeding depression was never an advantage in our study; moreover, our simulations did not predict that it should be. More studies are needed to investigate how specific conditions (e.g., effective population size, selfing rate, genetic architecture) affect such an advan-tage. A second unexpected result was the increase in non-genetic components of phenotypic variance, further impeding the response to selection. This effect, referred to as reduced genetic homeostasis, has already been observed [7] but has been overlooked as a potential cause of the low efficacy of adaptation in selfing populations and has usually been ignored in genetic models.

Our results suggest that adaptive challenges are less likely to be overcome by selfing than by outcrossing populations. This is relevant for many plants of agronomical interest, in which increased selfing rates are associated with strong selection dur-ing domestication [27]. Early cultivators may have benefited from the short-term positive effect of selfing on the response to direc-tional selection, allowing the rapid fixation of agronomically inter-esting traits. However, the long-term preservation of adaptive potential requires a dose of outcrossing among lines, as in wheat dynamical management strategies [28]. Similarly, the loss of pol-linators [29] may result in increased selfing rates [30] in plant populations that are at the same time facing climate change and biological invasions, possibly requiring rapid adaptation. Selfing certainly provides reproductive assurance, but our re-sults also suggest that it increases the risk of losing genetic vari-ation and failing to adapt when prolonged over more than a few generations.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures, three tables, and Supple mental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.11.015.

AUTHOR CONTRIBUTIONS

E.N., P.J., S.G., and P.D. designed the study, interpreted the results, and wrote the manuscript. E.N., A M., A.S., V.S., and P.D. performed the experiments and genetic analyses. E.N. and P.D. monitored the experimental evolution lines and analyzed the results.

ACKNOWLEDGMENTS

We thank B. Pélissié, T. Bataillon, H. Fréville, S. Maurice, and J. Ronfort for dis-cussions; two anonymous reviewers for comments; and the SMGE (CEFE) and Environmental Genomics platform (LabEx CeMEB) for support with the molec ular analyses. This work was a part of E.N.'s PhD project and was funded by the Agence Nationale de la Recherche (ESHAP, ANR 12 BSV7 0015, and AFFAIRS, ANR 12 ADAP 005 to P.D.) and by CNRS.

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