

LETTERS

Mutation load and rapid adaptation favour outcrossing over self-fertilization

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The tendency of organisms to reproduce by cross-fertilization despite numerous disadvantages relative to self-fertilization is one of the oldest puzzles in evolutionary biology. For many species, the primary obstacle to the evolution of outcrossing is the cost of production of males¹, individuals that do not directly contribute offspring and thus diminish the long-term reproductive output of a lineage. Self-fertilizing ('selfing') organisms do not incur the cost of males and therefore should possess at least a twofold numerical advantage over most outcrossing organisms². Two competing explanations for the widespread prevalence of outcrossing in nature despite this inherent disadvantage are the avoidance of inbreeding depression generated by selfing^{3–5} and the ability of outcrossing populations to adapt more rapidly to environmental change^{1,6,7}. Here we show that outcrossing is favoured in populations of *Caenorhabditis elegans* subject to experimental evolution both under conditions of increased mutation rate and during adaptation to a novel environment. In general, fitness increased with increasing rates of outcrossing. Thus, each of the standard explanations for the maintenance of outcrossing are correct, and it is likely that outcrossing is the predominant mode of reproduction in most species because it is favoured under ecological conditions that are ubiquitous in natural environments.

The vast majority of animals and plants reproduce by outcrossing, as opposed to selfing. This observation is puzzling, because theory suggests selfing enjoys several substantial fitness advantages over outcrossing^{8,9}. For example, selfing results in the production of offspring that are each capable of bearing offspring, whereas many outcrossing species produce males that do not bear offspring. This halving of the number of offspring-bearing progeny an individual can produce is known as the 'two-fold cost of males' and generates a large gap between the mating systems in numerical contribution, and thus fitness, over time¹. In addition to this inherent numerical advantage, selfing also efficiently reduces the mutation load over time by eliminating or 'purging' new harmful mutations by exposing them to natural selection via the production of homozygous offspring^{3,4}. However, if mutations are too numerous or have effect sizes that allow them to slip below the selection threshold, then deleterious mutations can accumulate unchecked within selfing lineages—something that should not happen in outcrossing populations of sufficient size^{5,10,11}. Further, any new adaptive mutations will tend to become trapped within different selfing lineages because the lack of outcrossing means that any mutations that arise within separate selfing individuals can not be incorporated into the same lineage or genome^{12,13}. In this way, selfing mimics the problems associated with asexual reproduction, with outcrossing providing a more effective means of recombination and thereby generating the genetic variation necessary to adapt to a novel environment⁷. To critically evaluate these theoretical predictions, it is necessary both to experimentally manipulate the mating system of a given species and to recapitulate

the evolutionary process under the specific conditions predicted to favour either selfing or outcrossing.

Here, we utilize experimental evolution in populations of *C. elegans* to test the benefits of outcrossing relative to selfing under conditions predicted to favour outcrossing. *C. elegans* populations are composed of males and hermaphrodites. Hermaphrodites reproduce through either self-fertilization or by outcrossing with males. Despite the potential for outcrossing with males, most *C. elegans* populations reproduce predominantly via selfing ('wild-type' outcrossing rates are generally less than 5%)^{14–19}. However, by incorporating one of two mating system altering mutations (*xol-1* and *fog-2*; refs 20 and 21, respectively), we generated both obligate selfing and obligate outcrossing populations, yielding three different outcrossing levels (obligate selfing, wild type, obligate outcrossing) within the same genetic background. These mutations were independently crossed into two separate genetic backgrounds (N2 and CB4856) with known differences in wild-type outcrossing rates¹⁹. Exposing these populations to two different novel selection environments—(1) elevated mutation rates coupled with a migratory barrier (Supplementary Fig. 1a) and (2) a virulent bacterial pathogen (Supplementary Fig. 1b)—allowed us to directly test theories advocating either deleterious mutations or adaptation to ecological conditions as the primary selective forces contributing to the prevalence of outcrossing as a means of sexual reproduction.

Selfing populations are thought to be able to purge new deleterious mutations as long as the mutations are not too frequent and their effect sizes are large enough to be exposed to selection^{3–5}. Indeed, even relatively small *C. elegans* populations have been shown to escape the most serious consequences of mutation accumulation, even when their mutation rate is increased tenfold²². However, outcrossing is predicted to slow the fixation of deleterious mutations with weak to moderate effect sizes. To explore these contrasting expectations, we subjected populations to the chemical mutagen ethyl methanesulphonate (EMS) every other generation at a level that increases individual mutation rate by approximately four times the natural rate. Populations exposed to the mutagen and populations maintained at natural mutation rates were reared and passaged within a novel environment (a Petri dish transected by a vermiculite barrier separating populations from their food source upon introduction to the dish) to impose strong selection and thereby facilitate the potential to purge deleterious mutations. We then tracked the subsequent evolution of 60 different populations for 50 generations under different combinations of mutation, mating system and genetic background.

Despite strong selection against deleterious mutations, obligate selfing populations fixed significantly more mutations than did the obligate outcrossing populations, as evidenced by the fact that the latter populations maintained fitness over the course of the experiment in spite of elevated mutation rates, whereas the selfing populations displayed a substantial decline in fitness (Fig. 1a; analysis of variance: $F_{1,481} = 456.15$, $P < 0.001$). The purging of deleterious mutations within selfing

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populations is easily overwhelmed by slight increases in mutation rate. In contrast, while outcrossing populations are more likely to accumulate segregating deleterious mutations¹¹, these mutations do not lead to an overall decline in mean fitness (Fig. 1a). The value of outcrossing is particularly evident in the wild-type populations, where outcrossing rates are free to vary as dictated by selection. The wild-type populations subject to elevated mutation rates exhibit increased levels of outcrossing (Fig. 1b; $F_{1,8} = 55.7$, $P < 0.001$), indicating that increased levels of outcrossing are favoured under these conditions.

Whereas fitness loss due to selfing is offset to a large extent by the intermediate amounts of outcrossing exhibited in the wild-type populations, obligate selfing CB4856 populations lose fitness over time even when maintained at their natural mutation rate (Fig. 1a; $F_{1,481} = 17.5$, $P < 0.001$). We replicated the deterministic loss of fitness in obligate selfing CB4856 populations under long term maintenance in more permissive laboratory conditions as well (20% fitness loss over 30 generations; $F_{3,71} = 9.85$, $P < 0.001$). Indeed, obligate selfing *C. elegans* populations would in general be expected to go extinct over the course of a few hundred generations²³. Several other studies have investigated the role that elevated mutation rates may play in maintaining males within partially selfing *C. elegans* populations, finding that increases in mutation can prolong the maintenance of males in the population, but at levels that are only slightly greater than wild type^{24,25}. Therefore, even partial outcrossing is a valuable, if not always sufficient, means of managing the influx of deleterious mutations.

As predicted, outcrossing ameliorates the fixation of deleterious mutations. However, alternative theories emphasize that outcrossing should enable a stronger and more rapid adaptive response to ecological conditions than selfing^{1,6,7,12,13}. Here, outcrossing (wild-type and obligate outcrossing) populations maintained at natural mutation

rates exhibited a significantly greater amount of adaptation than the obligate selfing populations after 50 generations of selection, regardless of genetic background (Fig. 1a; $F_{1,481} = 51.98$, $P < 0.001$). The observed rate of adaptation in the obligate outcrossing populations (0.34% increase in fitness per generation) is particularly impressive, because this adaptation occurred in near-isogenic lines over a span of only 50 generations. Thus, the majority of the adaptive response is likely to have been due to novel mutations.

To further test the ability of outcrossing to facilitate rapid adaptation, we exposed obligate outcrossing, wild type, and obligate selfing populations within a common CB4856 background to the bacterial pathogen *Serratia marcescens*. Several strains of *S. marcescens* elicit a pathogen avoidance behaviour from *C. elegans*²⁶, in addition to inducing the expression of a specific set of pathogen resistance genes following ingestion²⁷. *S. marcescens* 2170 is highly virulent when consumed by *C. elegans*, initially inducing an 80% mortality rate in our experimental regime (Supplementary Fig. 1b). Repeated exposures to *S. marcescens* therefore impose strong selection for either pathogen avoidance or resistance, or a combination of both responses. As a control, replicate populations were passaged on heat-killed *S. marcescens*. Before selection on *S. marcescens*, the experimental populations were mutagenized with EMS to generate standing genetic variation into the previously inbred experimental populations.

After 40 generations of exposure to *S. marcescens*, outcrossing populations adapted to the novel pathogenic conditions whereas the obligate selfing populations did not (Fig. 1c; $F_{1,80} = 245.79$, $P < 0.001$). The obligate outcrossing populations exhibited very rapid and substantial increases in fitness when exposed to *S. marcescens* (Fig. 1c; $F_{1,80} = 160.18$, $P < 0.001$). In addition, wild-type mating populations exposed to *S. marcescens* exhibited elevated outcrossing rates (Fig. 1d;

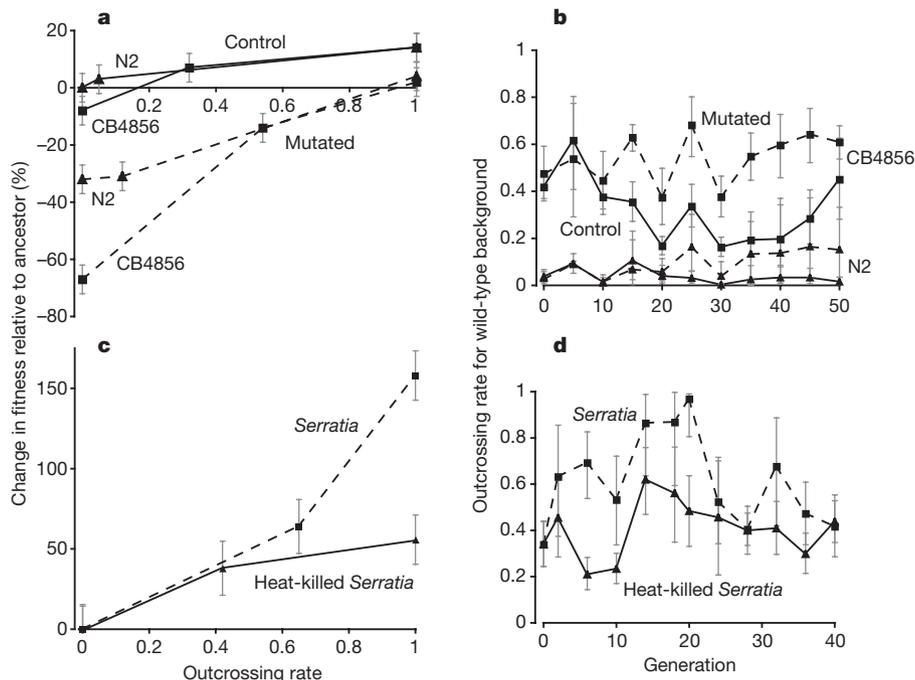


Figure 1 | Experimental test of the major theories of the evolution of outcrossing. **a**, Experimental populations (N2, triangles; CB4856, squares) with different outcrossing rates were exposed to a novel, challenging environment at either natural (solid lines) or elevated (4×; dashed lines) mutation rates for 50 generations. Percentage change in population mean fitness over time was assessed by comparing the competitive fitness of the ancestral population to that of the evolved population. Obligate selfing populations showed pronounced fitness decline in the face of elevated mutation rates (or even natural mutation rates in the case of CB4856). Both the rate of adaptation and resistance to mutational degradation increased with increasing levels of outcrossing. **b**, Within the wild-type outcrossing treatments, populations exposed to elevated mutation rates evolved higher outcrossing rates. **c**, Experimental populations with a CB4856 background

were mutated to generate genetic variation and then exposed to either the bacterial pathogen *S. marcescens* (dashed lines) or heat-killed *S. marcescens* control (solid lines) for 40 generations, then the percentage change in mean fitness was measured for each population. The outcrossing populations exhibited both rapid and substantial adaptation to the pathogen, but the obligate selfing populations failed to adapt. **d**, Populations exposed to *S. marcescens* evolved higher outcrossing rates within the wild-type outcrossing treatment. Thus, in keeping with theory, both the influx of deleterious mutations and adaptation to a novel environment favour outcrossing over selfing. Points represent the means of 5 replicate experimental evolution populations for **a** and **b**, and 6–7 populations for **c** and **d**. Error bars, ± 2 s.e.m. (errors calculated on arcsine-square-root transformed data for **b** and **d**).

$F_{1,5} = 27.2$, $P = 0.003$) and significantly greater fitness (Fig. 1c; $F_{1,80} = 9.29$, $P = 0.003$) than wild-type populations maintained on heat-killed *S. marcescens*, indicating that selection favoured outcrossing over selfing. In general, outcrossing first increased and then declined over the course of the experiment (approaching its maximum value of 1.0 after 20 generations), indicating that the change in outcrossing is an evolved rather than facultative response (Fig. 1d). Stronger selection imposed by *S. marcescens* and initial standing genetic variation enabled a much stronger evolutionary response (3.8% increase in fitness per generation) (Fig. 1c) than that observed in the first experiment (Fig. 1a). Overall, then, outcrossing enables more rapid adaptation to changing ecological conditions than does selfing.

The prevalence of outcrossing is something of an evolutionary puzzle, given the inherent advantages of self-fertilization. This work provides the first (to our knowledge) experimental tests of the selective pressures favouring the evolution and maintenance of outcrossing. We have demonstrated that outcrossing impedes the fixation of deleterious mutations and facilitates rapid adaptation relative to selfing, such that outcrossing is at least conditionally favoured by selection. Similar results have been observed in accelerated rates of evolutionary change in sexual versus asexual populations^{28,29}. Although we cannot directly address the question of the origin of selfing and outcrossing in our experiments, overall levels of outcrossing increased in our wild-type treatments in which selfed and outcrossed offspring were competing within the same population (Fig. 1b, d). These results support the idea that obligate selfing may often be an evolutionary dead-end, in which species that evolve obligate selfing are ultimately doomed to extinction owing to an inability to respond to changing environmental conditions⁶.

The fact that obligate outcrossing yielded a much larger response than natural outcrossing rates is something of a surprise, because it is thought that moderate amounts of outcrossing are sufficient to escape the problems associated with obligate selfing¹¹. One additional feature of this system that has not been previously considered, however, is that an increase in the frequency of males within a population also increases the opportunity for sexual selection, which has been shown to reduce the overall genetic load within a population³⁰. Males therefore play multiple roles within these populations, both for enhancing genetic exchange across generations and increasing the efficacy of natural selection within generations. Mutation, changing environmental conditions, and pathogens are nearly ubiquitous selective pressures for many organisms, which probably explains outcrossing's relative prevalence in nature.

METHODS SUMMARY

We conducted two large-scale experimental evolution studies. First, we exposed obligate outcrossing, wild-type mating, and obligate selfing populations with approximately 500 individuals apiece to 0.5 mM EMS every other generation for 50 generations. These mutated populations, in addition to replicate populations maintained at natural mutation rates, were passaged each generation in a selective novel environment (Supplementary Fig. 1a). Second, we exposed obligate outcrossing, wild-type mating, and obligate selfing populations composed of approximately 500 individuals to *S. marcescens* (Supplementary Fig. 1b) for 40 generations while exposing replicate populations to heat-killed *S. marcescens* as a control. These populations were exposed to 10 mM EMS for four generations before selection as a means of inducing genetic variation. We used a competitive fitness assay to measure the change in fitness for each experimental population relative to its ancestor before selection. The competitive fitness assays were conducted within the context of the selective environment and the assay was conducted simultaneously on the experimental population and the previously frozen ancestral population. Fitness was determined by mixing each population (experimental and ancestral) with a GFP-marked tester strain at a 50:50 ratio. After passaging the worms in the relevant selective environment, the GFP ratio of the offspring was calculated and used to estimate fitness.

Received 13 August 2009; accepted 11 September 2009.

Published online 21 October 2009.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank S. Scholz, A. Ohdera and J. Chiem for logistical help, S. Katz for providing the *S. marcescens* 2170 strain, and J. Thornton for use of laboratory space and equipment. We also thank B. Cresko, C. Lively, J. Thornton and the members of the Phillips and Cresko laboratories for comments and discussion pertaining to this work. Funding was provided by NSF grants DEB-0236180, DEB-0710386 and DEB-0641066, and an NIH Genetics Fellowship awarded to L.T.M. Some nematode strains used in this work were provided by the *Caenorhabditis* Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR).

Author Contributions L.T.M. and P.C.P. designed the experiments. L.T.M. and M.D.P. performed the experiments. L.T.M. and P.C.P. analysed the data. L.T.M. and P.C.P. wrote the paper.

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