Adaptation to temporally fluctuating environments by the evolution of maternal effects

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Abstract

Most organisms live in ever-challenging temporally fluctuating environments. Theory suggests that the evolution of anticipatory (or deterministic) maternal effects underlies adaptation to environments that regularly fluctuate every other generation because of selection for increased offspring performance. Evolution of maternal bet-hedging reproductive strategies that randomize offspring phenotypes is in turn expected to underlie adaptation to irregularly fluctuating environments. Although maternal effects are ubiquitous their adaptive significance is unknown since they can easily evolve as a correlated response to selection for increased maternal performance. Using the nematode *Caenorhabditis elegans*, we show the experimental evolution of maternal provisioning of offspring with glycogen, in populations facing a novel anoxia hatching environment every other generation. As expected with the evolution of deterministic maternal effects, improved embryo hatching survival under anoxia evolved at the expense of fecundity and glycogen provisioning when mothers experienced anoxia early in life. Unexpectedly, populations facing an irregularly fluctuating anoxia hatching environment failed to evolve maternal bet-hedging reproductive strategies. Instead, adaptation in these populations should have occurred through the evolution of balancing trade-offs over multiple generations, since they evolved reduced fitness over successive generations in anoxia but did not go extinct during experimental evolution. Mathematical modelling confirms our conclusion that adaptation to a wide range of patterns of environmental fluctuations hinges on the existence of deterministic maternal effects, and that they are generally much more likely to contribute to adaptation than maternal bet-hedging reproductive strategies.

Introduction

In environments that fluctuate in a regular way across generations, such as seasonal climate changes bivoltine insects experience, the maternal environment is a reliable cue for the offspring environment. Natural selection in these circumstances creates covariances between the genes individuals pass on and the environments their mothers have experienced [1,2], which can lead to the evolution of transgenerational plasticity or anticipatory maternal effects [3-6]. Hereafter, we refer to "deterministic" maternal effects when the mother's phenotype in a given environment has a consistent effect on the offspring phenotype in spite of offspring genotype. Conversely, when the environment fluctuates irregularly across generations, such as the erratic water availability that xeric organisms may face in deserts, the maternal environment is not a reliable cue of the offspring environment. While deterministic maternal effects cannot evolve in this context, bet-hedging reproductive strategies can be favored whereby mothers' produce offspring with a randomized mix of phenotypes, ensuring that at least

50 some will be able to survive and reproduce no matter which environment they
51 experience [7,8]. Bet-hedging strategies, here called "randomizing" maternal effects, are
52 expected to be adaptive when they increase long-term growth rates [9-12].

53   Maternal effects, particularly deterministic maternal effects, are common in a
54 wide variety of fungi, plants and animals [13-18]. It is, however, questionable if
55 maternal effects are adaptive [14,18-21], because they may simply evolve as a
56 correlated response to selection for increased maternal performance [22]. If maternal
57 effects are adaptive in fluctuating environments they must evolve because of selection
58 for increased offspring performance, despite potential selection on maternal traits. The
59 evolution of maternal effects is further expected to be correlated with the evolution of
60 compromised maternal performance [6,24,25].

61   Taking advantage of the *Caenorhabditis elegans* mother-offspring conflict
62 between survival in hyperosmotic and anoxic environments as our paradigm [26], we
63 here show that the evolution of maternal glycogen provisioning underlies experimental
64 adaptation to a novel and regularly fluctuating anoxia environment because of selection
65 for improved embryo hatchability in anoxia. We also demonstrate that the evolution of
66 maternal effects is detrimental to the mothers. While populations experiencing
67 predictable changes in rearing anoxia evolved deterministic maternal effects,
68 populations facing an irregularly fluctuating anoxia environment failed to evolve
69 maternal bet-hedging reproductive strategies, instead relying on the evolution of long-
70 term transgenerational effects for adaptation. Mathematical modelling supports our
71 conclusion that, once evolved, deterministic maternal effects and associated fitness
72 benefits may underlie adaptation to a wide range of temporally fluctuating
73 environments.

74

Results and Discussion

Hyperosmotic maternal survival hinders offspring anoxia survival. When
77 hermaphrodites of the N2 genotype of *C. elegans* experience an osmotic stress their
78 embryos show much reduced hatchability to the first larval stage if facing an anoxia
79 stress [26]. This maternal effect is due to a metabolic trade-off between the
80 hermaphrodites' ability to produce glycerol during growth from larval stages to maturity
81 and the ability to provision their embryos with glycogen [26,27]. We confirmed for
82 several genotypes derived from our lab-adapted population that hermaphrodites reared
83 in high NaCl concentrations resulted in a survival cost to their anoxia-exposed embryos
84 [28,29] (Fig. S1). We therefore asked if adaptation to fluctuating oxygen levels across
85 mother-offspring generations could be achieved by the evolution of strategic glycogen
86 provisioning.

87   To address this question, we first evolved our lab-adapted population to high
88 NaCl concentrations [30], so that evolution under fluctuating oxygen levels across
89 mother-offspring generations would not be confounded with adaptation to a

90   hyperosmotic challenge. A single salt-adapted population was then used as the ancestor

91   for all experimental populations in the present study. This ancestor population had

92   appreciable standing genetic diversity although males were present at a low frequency

93   so that most reproduction was done by self-fertilization [30] (Fig. S2). Further, this

94   ancestor population showed a marked reduction in fitness when embryos were exposed

95   to anoxia, measured as the per capita growth rate (Fig. 1A), independently of the

96   maternal hatching environment, indicating that it was well adapted to normoxia but had

97   a substantial potential for selection on embryo hatchability in anoxia (Fig. 1B; Linear

98   mixed effects modelling LMM, followed by planned Tukey t test with corrected

99   Kenward-Roger degrees of freedom: $t_{93.7}$ P<0.001; there was no interaction between

100   offspring and maternal hatching environments).

101

Experimental evolution under fluctuating anoxia hatching environments.

103   Experimental evolution to fluctuating oxygen levels across mother-offspring

104   generations was done for 60 generations under two regimes, each four-fold replicated

105   (Fig. 1C). The "predictable" regime imposed alternating normoxia and anoxia

106   conditions every other generation. Specifically, we designed an environmental sequence

107   where the probability of mothers and offspring sharing the same hatching environment

108   was 0.05, while making sure that the total number of normoxia versus anoxia

109   generations were equal. Similarly, the "unpredictable" regime had an equal number of

110   normoxia and anoxia generations but two environmental sequences were chosen where

111   the probability of mothers and their offspring sharing the same hatching environment

112   was 0.45. This design ensures that non-maternal effect genotypes can perform equally

113   well regardless of the order of the anoxia-normoxia generations in the experiment. A

114   "constant" control regime was characterized by 30 consecutive generations of anoxia,

115   also four-fold replicated. Adaptation in this regime was expected to occur through the

116   evolution of increased embryo hatchability in anoxia, or increased hermaphrodite

117   fecundity following anoxia, regardless of the existence of maternal effects. As was the

118   case during previous laboratory adaptation [28] and previous salt-adaptation [30], all 12

119   experimental populations were maintained under discrete time and non-overlapping 4

120   day life-cycles at fixed larval L1 stage to reproductive adulthood density of $N=10^4$, such

121   that most adaptation should have occurred from the sorting of standing genetic diversity

122   [31,32].

123   After experimental evolution, the predictable populations showed a considerable

124   increase in fitness when hermaphrodites experienced normoxia and their embryos

125   experienced anoxia (Fig. 1D; $t_{15.7}$ P<0.001), but showed no change in fitness when

126   hermaphrodites experienced anoxia and their embryos experienced normoxia.

127   Interestingly, there were also changes in fitness in the two mother-offspring

128   environments that were largely absent from the sequences imposed on the predictable

129   populations during experimental evolution. Under two successive generations of

130   normoxia fitness increased ($t_{10.9}$ P=0.03), while under two successive generations of

131   anoxia fitness decreased ($t_{11.2}$ P=0.02). Because these two mother-offspring

132   environmental transitions were not under selection during experimental evolution, they
133   must have evolved as correlated effects.

134   In contrast to the predictable populations, the unpredictable populations did not
135   improve their fitness in any combination of mother-offspring hatching environments
136   after experimental evolution. In fact, and similarly to the predictable populations, the
137   unpredictable populations showed a significant fitness reduction when exposed to
138   anoxia for two consecutive generations ($t_{18.5}$ P=0.04).

139   The constant populations evolved increased fitness in anoxia after 30 generations
140   of continuous exposure to anoxia, regardless of the maternal hatching environment
141   (anoxia: $t_{7.2}$ P<0.01; normoxia: $t_{8.5}$ P<0.01). Because our design exposed all
142   experimental populations to the same number of anoxia generations, we can therefore
143   rule out the possibility that lack of genetic variation, small genetic effects, or small
144   population sizes limited the scope for adaptation to anoxia.

145

146   **Evolution of deterministic maternal effects trades-off with maternal fecundity.** Are
147   these fitness responses of the predictable populations consistent with the evolution of
148   deterministic maternal effects? There are two components of life-history that could be
149   involved in adaptation to fluctuating anoxia: changes in the fecundity of adult
150   hermaphrodites (a direct effect) and changes in embryo hatchability due to maternal
151   glycogen provisioning (a deterministic maternal effect).

152   In the predictable populations, we found that adult hermaphrodites who
153   experienced anoxia early in life had reduced fecundity (Fig. 2A; $t_{3.6}$ P=0.03), and those
154   that experienced normoxia had similar fecundity ($t_{3.9}$ P=0.4), when compared to
155   ancestral hermaphrodites. Remarkably, a clear deterministic maternal effect is seen in
156   the hatchability response in that hermaphrodites exposed to normoxia early in life
157   produce embryos with increased hatchability in general, with a larger response under
158   anoxia (Fig. 2B; offspring anoxia: $t_{14.5}$ P<0.01, offspring normoxia: $t_{12.9}$ P=0.03). These
159   results thus parallel the fitness results and provide an explanation for the observed
160   correlated response of an increase in normoxia-normoxia fitness (an environmental
161   sequence that the predictable populations did not face during experimental evolution).
162   Specifically, evolution of increased embryo hatchability in normoxia resulted in
163   increased fitness in normoxia. These results additionally demonstrate the evolution of a
164   trade-off between embryo survival under anoxia and adult hermaphrodite fecundity after
165   hatching under anoxia, in line with theoretical expectations [6,24,25].

166

167   **Evolution of maternal provisioning of glycogen to offspring.** We directly confirmed
168   the hypothesis that the deterministic maternal effect that evolved in the predictable
169   populations was mediated by changes in strategic maternal glycogen provisioning [26].
170   Image analysis of iodine-stained oocytes and early embryos within hermaphrodites
171   showed the evolution of increased glycogen content only when they experienced

172   normoxia early in life (Fig. 2CD; oocytes: $t_{8.8}$ P<0.01, embryos: $t_{7.6}$ P<0.001; see also
173   Fig. S3). Surprisingly, both oocyte and embryo glycogen content was reduced relative
174   to the ancestor population in anoxia-exposed hermaphrodites (oocytes: $t_{8.2}$ P=0.03,
175   embryos: $t_{11.3}$ P<0.01), suggesting, like fecundity, a trade-off between embryo anoxia
176   survival and hermaphrodite glycogen provisioning after being reared under anoxia. The
177   evolution of reduced glycogen provisioning appears to be inconsequential, however,
178   since embryo hatchability in normoxia --the environment the embryos produced by
179   hermaphrodites hatched under anoxia would normally face-- did not change during
180   experimental evolution.

181

182   **Adaptation to irregularly fluctuating environments does not involve maternal bet-**
183   **hedging strategies.** Having shown the evolution of a deterministic maternal effect in
184   the predictable populations, we address whether there was evolution of a randomizing
185   maternal effect in the unpredictable populations. In temporally fluctuating environments
186   the appropriate measure of adaptation is the geometric mean of environment-specific
187   fitness [9,10,12]. The presence of maternal effects (deterministic or randomizing) does
188   not change this principle, but does require averaging over the frequencies of the
189   pairwise environmental transitions experienced by the population during its history.
190   With uncorrelated environmental variation, reproductive strategies that reduce
191   generation-to-generation variation in maternal performance and increase the geometric
192   mean of fitness are known as bet-hedging strategies [7,8,18]. If there was evolution of
193   maternal bet-hedging in the unpredictable populations then their geometric mean
194   fitness, averaged over the four pairwise mother-offspring hatching environments, should
195   have improved relative to the ancestor population.

196   We used a two-generation model of maternal effect carryover to estimate the
197   geometric mean fitness of the unpredictable populations and found no evidence that it
198   changed with experimental evolution (Fig. 3A; t_6 P=0.19). The predictable populations,
199   as expected, increased their geometric mean fitness across the two environmental
200   transitions they experienced during their history (t_6 P<0.01). Numerical simulations
201   indicate that this lack of response in the unpredictable populations is not due to the fact
202   that we measured the geometric mean fitness of genetically variable populations [8,11]
203   (Fig. 3BC; Fig. S4). Further, since unpredictable populations did not go extinct during
204   experimental evolution, another component of the long-term fitness must have increased
205   enough to compensate the observed reduction in anoxia survival over two successive
206   generations (vid. Fig. 1D). Multi-generational carryover effects are indeed expected to
207   be adaptive in irregularly fluctuating environments [25,33-36], and there was scope for
208   their evolution since the unpredictable populations faced a set of environmental
209   transitions that were correlated over the long-term.

210

211   **Which maternal effects underlie adaptation to fluctuating environments?** To better
212   understand how the sequence of environmental transitions determines the course of

213   evolution we developed mathematical models to calculate the probability that a
214   genotype conferring maternal effects would invade the ancestral population [11,37] (see
215   Methods). We considered a scenario with two offspring phenotypes, one that had higher
216   fitness under anoxia and the other that had higher fitness under normoxia. Deterministic
217   maternal effects were defined by a genotype that always produced offspring with the
218   phenotype suited to the environment that the mother did not experience. The
219   randomized maternal effects genotype produced a brood of offspring with a fixed
220   fraction of each phenotype, with the fraction chosen in order to maximize the geometric
221   mean fitness of the bet-hedging strategy.

222   We found that the deterministic maternal effect can only evolve if the probability
223   of switching environments is above 0.5, and increases as the probability of switching
224   goes up (Fig. 4A). The maternal bet-hedging strategy has a constant benefit relative to
225   the ancestor for switching rates of less than 0.5, and is outcompeted by the deterministic
226   maternal effect strategy when the probability of switching is large. The maximum
227   probability of fixation for the randomizing bet-hedging strategy is only about 1/10 that
228   of the deterministic maternal effect, even if the same environment-specific phenotypic
229   effects were parameterized in both kinds of maternal effects.

230   Experimental evolution results are thus consistent with our theoretical analysis,
231   particularly because adaptation caused by maternal effects was only observed in the
232   predictable populations. To illustrate that deterministic maternal effects are potentially
233   adaptive in a range of patterns of environmental fluctuations, we used the evolved
234   predictable populations' two-generation fitness values to calculate the geometric mean
235   fitness for arbitrary sequences of fluctuating oxygen level hatching environments (Fig.
236   4B). The simulations show that the evolved genotypes would perform better than the
237   ancestral genotypes even in environmental fluctuation regimes that were not used in
238   experimental evolution. This suggests that the evolution of deterministic maternal
239   effects and correlated fitness benefits unlocked an adaptive potential that was not
240   accessible to the ancestral population. We conclude that adaptation to regularly
241   fluctuating environments may improve a populations' future prospects of withstanding a
242   wide array of environmental fluctuation patterns.

243

Conclusion

245   Our analysis demonstrates that deterministic maternal effects, and possibly multi-
246   generational carryover effects, can underlie adaptation to temporally fluctuating
247   environments. We find much less support that maternal bet-hedging reproductive
248   strategies randomizing offspring phenotypes make a strong contribution to adaptation.
249   With anthropogenic activities increasingly contributing to more extreme and irregular
250   climate fluctuations [38], it will be important to understand if deterministic maternal
251   effects are also key in maintaining biodiversity and in preventing extinction [39].

252

Materials and Methods

Salt-anoxia survivorship in the lab-adapted population. Four inbred lines of a lab-adapted population, A6140, were derived by 12 generations of enforced self-fertilization as previously described [29] (A6140L126, A6140L142, A6140L188, A6140L244), and stocks frozen at -80ºC. On the third generation after thawing and passaging under the standard lab conditions, first stage larvae (L1s) from each of the inbred lines were seeded in either 25mM NaCl or 300mM NaCl standard NGM-lite plates with *E. coli* [28]. After 96h, fifty embryos were hand-picked and transferred to 6cm Petri dishes with 25mM NaCl NGM-lite and a 10uL drop of *E. coli*, and then placed under normoxia or anoxia conditions for 16h (see below). After 96h, the number of adults was scored. For each inbred line and maternal-offspring treatment, 5 replicates were done. For statistical analysis, ANOVA was done with inbred line (4 levels) and maternal-offspring treatment (4 levels) as fixed factors. F-tests were employed to test for significance of main factors and interaction. Post-hoc Tukey t-tests were then done to contrast maternal-offspring treatments. In a separate assay, line A6140L244 was similarly assayed for anoxia survivorship from embryo to adulthood, when hermaphrodites from the maternal generation were reared from 24h to 96h of the life-cycle in 25mM, 100mM, 200mM, 225mM, 250mM, 275mM and 300mM NaCl NGM-lite plates. Twenty replicates per treatment were done.

Environmental sequences. The predictable environmental sequence of anoxia-normoxia generations was designed such that the probability of repeating the same oxygen level hatching conditions across two generations was of 0.05, and the frequency of anoxia and normoxia events was 0.5 for a total of 60 generations. The two unpredictable sequences (#11, #19) were designed such that the probability of repeating the same oxygen level hatching conditions across two generations was of 0.45 and across three generations of 0.5. The frequency of anoxia and normoxia generations was 0.5 for a total of 60 generations. The constant environment was characterized by 30 consecutive generations of anoxia. QBasic64 v0.954 was used to design the sequences.

Experimental evolution. All populations were derived from a high salt-adapted population [30] (GA250), which in turn was derived from a lab-adapted population [28,29] (A6140). Frozen GA250 samples with $>10^4$ individuals were thawed from frozen -80ºC stocks and cultured for one generation for number expansion, before replicate population derivation. Predictable, unpredictable and constant populations were designated Pi, Ui and Ci, respectively, with *i* standing for 1 to 4 replicate number. During experimental evolution a random number was assigned to each population to avoid potential bias. Environmental sequences #11 and #19 were each replicated in two populations. Following our standard laboratory environment [28,30], populations were kept in ten 9 cm Petri plates with 28mL of solid NGM-lite agar media (Europe Bioproducts) covered by an overnight grown lawn of HT115 *E. coli* food. NaCl concentration in the NGM-lite media was of 305mM (1.78% w/v)[30]. At 24h±2h of the life cycle, each population was seeded with 1,000 first larval staged (L1) individuals in each of the ten Petri plates. After growth to maturity for 66h±1h at constant 20ºC and

295   80%RH in controlled incubators (Fitoclima D1200, ARALAB), all ten plates were
296   mixed and worms harvested with 5mL M9 isotonic solution and then exposed to 1M
297   KOH: 5% NaOCl "bleach" solution for 5min±15sec, to which only embryos survive.
298   After repeated washes with M9, 200μL containing embryos (and larval and adult debris)
299   were transferred to 25mM NaCl NGM-lite plates, without *E. coli*. These plates were
300   then placed inside 7L polycarbonate boxes with rubber clamp sealed lids (AnaeroPack,
301   Mitsubishi Inc.). Within these boxes an anoxic embryo hatching condition was imposed
302   by placing two GasPak^{TM} EZ sachets (Becton, Dickinson and Company). These sachets
303   contain inorganic carbonate, activated carbon, ascorbic acid and water, which after two
304   hours of activation will produce an anaerobic/anoxic atmosphere inside the boxes (<1%
305   O_2, ≥13% CO_2; according to the manufacturer). At every generation, in all boxes,
306   anoxia conditions were confirmed by placing two BBL^{TM} Dry Anaerobic Indicator
307   strips (Becton, Dickinson and Company). To prevent drying, paper towels with 20mL
308   ddH$_2$0 were placed within each box. For normoxia hatching conditions, the sachets were
309   not used and an additional 60mL ddH20 was placed inside each box. After 16h, hatched
310   L1s were washed off the plates with 3-5mL M9 to a 15mL Falcon tube, adult debris
311   removed after centrifugation at 200rpm and, live L1 density estimated under a Nikon
312   SMZ1500 dissection scope in five 5uL M9 drops at 40x magnification. While
313   estimating, tubes were kept in a shaker at 120rpm (Lab. Companion SK-600) inside the
314   incubators. 6h±1h after, the appropriate M9 volume for 1,000 live L1s was placed in
315   fresh NGM-lite plates to complete one life cycle.

316   **Fitness assays.** P1-4 and U1-4 from generation 60 and C1-4 from generation 30 were
317   measured for fitness alongside the ancestral GA250 population in two-generation long
318   assays. Frozen -80°C stocks ($n>10^3$) were thawed and reared in a common environment
319   for two generations before assaying. On the third generation, adults were washed off the
320   plates, treated with the "bleach" solution and their embryos were exposed to normoxia
321   or anoxia to constitute the maternal hatching assay environment. 24h later, 1,000 of
322   surviving L1s were seeded in each of 5 Petri dish plates, allowed to grow to adulthood,
323   treated with the "bleach" solution and their embryos were exposed in a factorial fashion
324   to normoxia or anoxia to constitute the offspring hatching assay environment. After 16h
325   of exposure to the corresponding hatching environment, worms were washed off the
326   plates with 3-5mL of M9 to a 15mL Falcon tube before the total number of surviving
327   L1s was estimated by considering the total volume of the M9 solution and counting the
328   number of live L1s in ten to fifteen 5μL drops. The total number of live L1s was then
329   divided by 5,000 to calculate the maternal L1 to offspring L1 growth rate. This ratio
330   defined fitness in our two-generation paradigm as the per capita growth rate. The assays
331   were done in 16 blocks, defined by different thawing dates of the samples. In each block
332   the ancestral GA250 population was included. Per population and hatching
333   environmental treatments there were 3 replicate measurements.

334   **Fecundity and hatchability assays.** These assays were similar in design to the fitness
335   assays, with two-generation exposure to all four mother-offspring oxygen hatching
336   environment combinations. P1-4 from generation 60 and the ancestor GA250 ($n>10^3$)

337 were thawed and grown in parallel for two generations before assaying. For each
338 replicate measurement, 1,000 live L1s after exposure to normoxia or anoxia maternal
339 hatching environments were grown in 6 to 10 plates at a density of 1,000. Adult worms
340 were washed off and treated with the "bleach" solution. In contrast with the fitness
341 assays, after the "bleach" treatment the dead adults were removed from the M9 Falcon
342 tube after centrifugation at 200rpm. The number of embryos was then scored in ten 5μL
343 M9 drops to estimate the total number of embryos in the M9 volume after the "bleach"
344 treatment. This total number was then divided by the total number of adults to calculate
345 the per capita fecundity. The embryo-only M9 solution was equally divided within 2h
346 after the "bleach" treatment, centrifuged at 1,800rpm. The pellet containing the embryos
347 was then exposed to anoxia or normoxia hatching conditions. After 16h the density of
348 live L1s was estimated. Hatchability was then calculated as the ratio of live L1s over
349 embryo number. The assays were done in two thawing time blocks, for 3 replicate
350 measurements per population and hatching condition.

351 **Glycogen content assays**. Adult hermaphrodites from generation 60 P1-4 and GA250
352 populations were assayed for glycogen content in the oocytes and embryos inside the
353 body. After exposure to a normoxia or an anoxia hatching environment, adult
354 hermaphrodites were subjected to fixation. This was done by suspending them in a
355 solution of 700 μL of absolute ethanol (VWR Scientific) with 200 μL of glacial acetic
356 acid (Carlo Erba) and 100 μl of concentrated formalin (Sigma-Aldrich) for 90 minutes.
357 Serial dehydration was then done by sequentially re-suspending worms in 70%, 90%
358 and absolute ethanol. Samples were stored at -20°C. Frozen hermaphrodites were
359 sequentially rehydrated in absolute, 90% and 70% ethanol, before being re-suspended in
360 the M9 solution. Hermaphrodites were then transferred to a glass slide topped with a
361 thin agar pad (5% noble agar; Becton, Dickinson and Company) with a pipette.
362 Hermaphrodites from the ancestral and one of the predictable populations were assayed
363 simultaneously by placing them on the same agar pad. For staining, each glass slide was
364 placed upside down over the mouth of a bottle with 100g of iodine (I$_2$, ACS≥99.8%
365 solid; Sigma-Aldrich) for 120 seconds [26,27]. Within 40 minutes, photographs were
366 taken at 630x magnification under differential interference contrast (DIC) settings in a
367 Zeiss Axioskop2 microscope coupled to a monochromatic CCD camera (Hitachi Denshi
368 ltd.). The DIC settings for the microscope and camera were kept identical across all
369 glass slides. For each hermaphrodite, 2-3 images were taken to cover developing
370 embryos and/or oocytes at the anterior half of the body from the vulval region. Similar
371 images were obtained for unstained samples. The images were not manipulated for
372 contrast of grayscale levels. For analysis we used ImageJ 1.46r, with the first three
373 oocytes from the spermatheca, or all visible embryos, being manually delineated and the
374 mean pixel intensity over the measured area recorded. For all the images, the mean pixel
375 intensity of agar pad was also obtained to account for the decay of staining with time
376 and/or other non-specific staining variation across images. The ratio of the mean pixel
377 intensity of the oocytes/embryos over the mean pixel intensity of the agar pad was used
378 as the raw data for statistical analysis. Size was taken as the perimeter in pixels of the

379   individually delineated oocytes. The assays were run in one block, for 3 to 8 replicate

380   measurements per population and hatching oxygen condition.

381   **Statistical data analysis.** Fitness data in the ancestral population was analyzed with a

382   linear mixed effects model (LMM) and REML estimation methods [40], taking maternal

383   and offspring hatching conditions as fixed two level factors, and block as a random

384   factor. A preliminary model revealed that residuals did not follow normality (as tested

385   with the Shapiro-Wilk test), and thus outliers were removed through the inspection of

386   Quantile-Quantile Plots. Contrasts were then performed among the mother-offspring

387   hatching conditions, using post-hoc Tukey t-tests while estimating the effective number

388   of degrees of freedom with the Kenward-Roger (KR) approximation. For evolutionary

389   responses, we transformed the data from the derived populations by taking the ratio to

390   the ancestral mean value per block. Transformed data was analyzed by LMM, taking

391   replicate population as the random factor in order to account for the effects of genetic

392   drift and other historical accidents. Fitness response data was log-transformed to solve

393   normality issues, with the least-square estimates presented being the back-transforms.

394   As above, the KR approximation was used to estimate degrees of freedom in planned

395   Tukey t-tests. For fitness responses, we first modelled experimental evolution regime (2

396   levels, predictable and unpredictable) and maternal-offspring hatching conditions (4

397   levels) as the fixed factors. Generation 30 fitness data from the constant populations was

398   analyzed separately since the extent of genetic drift in them was halved relative to

399   predictable/unpredictable populations. Including the constant populations in the first

400   model would underestimate the random replicate population effects. For fecundity,

401   glycogen content and oocyte size responses, we modelled the maternal hatching

402   environment (two levels) and for the hatchability responses maternal-offspring hatching

403   environments (with four levels) as the fixed factors. For the geometric mean fitness

404   responses, fitness values in normoxia-anoxia and in anoxia-normoxia transitions in

405   predictable populations and in all four pairwise transitions for unpredictable populations

406   were modelled together with ancestral fitness values by LMM, taking block as a random

407   factor. The geometric mean of the estimated mean least-squares were then calculated,

408   with predictable and unpredictable responses taken as the ratio to the ancestral in an

409   ANOVA with experimental regime as a fixed factor. For all models, significant

410   evolutionary responses were inferred when the estimated effects deviated from one (the

411   fixed ancestral state), as tested with Student t-tests and, if needed, KR estimated degrees

412   of freedom. Least-square mean and error estimates are presented in all plots. The

413   packages *stats*, *lme4*, *lsmeans* and *pkbrtest* in R were used for computation [41].

414   **Genotyping and genetic diversity analysis.** Immature hermaphrodites were

415   handpicked from GA250 and generation 60 U1-4 populations and their gDNA collected

416   using prepGEM Insect kit (ZyGEM). Bi-allelic single-nucleotide polymorphisms

417   (SNPs) were chosen based on the pooled genome sequence of the A6140 population

418   (unpublished data). SNPs were genotyped with the iPlex Sequenom™ technology

419   [28,42]. WS200 genome version was used for the oligonucleotide design and SNP

420   physical position (www.wormbase.org). SNPs in chromosome I and II were genotyped

421   in 48 GA250 individuals and in 16 individuals from each of the U1-4 populations,
422   chromosomes III-IV and separately V-VI were genotyped with similar sample sizes.
423   SNPs for which the assay failed in more than 30% of the samples were eliminated. This
424   was followed by elimination of individuals with more than 10% of undetermined allele
425   identity, and finally elimination of SNPs with failed assays in more than 15% of the
426   remaining individuals across populations. A total of 478 SNPs were used for analysis
427   (chr. I, 4.5 SNPs/Mbp; chr.II, 6.1 SNPs/Mbp; chr. III, 6 SNPs/Mbp; chr. IV, 4
428   SNPs/Mbp; chr. V, 4.8 SNPs/Mbp; chr. VI, 6.9 SNPs/Mbp). Within-population fixation
429   indices (Fis) were calculated as 1 minus the ratio of observed (Ho) to expected
430   heterozygosity levels under random mating [42].

431   **Mathematical modelling.** We took two modelling approaches, one based on a general
432   model of maternal effects that we used to predict when maternal effects are likely to
433   evolve and another based on the empirically observed fitness responses that we used to
434   predict how the evolved populations would respond to novel environmental sequences.
435   All computations were done using Wolfram Mathematica 9.

436   In the general model we calculated the geometric mean fitness of genotype k as
437    $G_k = \exp(\sum p(i,j)\log(w_k(i,j)))$, where G_k represents the geometric mean fitness of
438   genotype k, $p(i,j)$ is the frequency of transitions from hatching environment i to
439   hatching environment j, and $w_k(i,j)$ is the reproductive output of a genotype k
440   individual developing in environment j whose mother experienced hatching
441   environment i. We model maternal effects by assuming that there are two possible
442   offspring phenotypic states, normoxia adapted and anoxia adapted. Without loss of
443   generality, we assigned the normoxia adapted phenotype relative fitness values of 1, so
444   that $w_N(i,j) = 1$. We assigned the log fitness of the other anoxia phenotype by drawing
445   from an exponential distribution with parameter γ so that fitness in anoxia was
446   increased and fitness in normoxia was decreased. A value of $\gamma = 2.0$ means that the
447   average advantage of the anoxia adapted phenotype in anoxia conditions is about 2 fold,
448   on the order of the fitness increase we observed following experimental evolution. We
449   assumed for simplicity but without loss of generality that the ancestral phenotype, the
450   normoxia adapted phenotype, had higher geometric mean fitness than the anoxic
451   phenotype.

452   We then used the geometric mean to calculate the probability of fixation of
453   either a genotype conferring a Deterministic Maternal Effect (DME) or a Randomizing
454   Maternal Effect (RME). The ancestral state was assumed to have no maternal effect and
455   therefore produce a constant phenotype adapted to normoxia conditions. Under DME,
456   mothers alter their offspring phenotype based on their own hatching environment.
457   Under RME, mothers produce a fraction q of offspring with the anoxia adapted
458   phenotype and $(1-q)$ with the normoxia phenotype. We assumed q was tuned to
459   maximize the geometric mean fitness of the genotype, an assumption that favors RME.
460   Given that experimental populations were maintained in discrete time non-overlapping
461   generations, the probability of fixation of an invading genotype (labelled U_{DME}
462   and U_{RME}, respectively) was calculated using M. Kimura's approximation of the

463 Wright-Fisher process [11,43]. In line with other evolution experiments from standing
464 genetic diversity, we further assumed that effective populations sizes were one order of
465 magnitude lower than the experimental census sizes [32,42]. We defined the effective
466 selection coefficient for use in Kimura's equation as the geometric mean of the
467 reproductive output of a genotype, which is expected to be a good approximation so
468 long as the probability a genotype goes extinct due to selection is not abnormally high
469 in early generations [37,44]. Because our populations had standing genetic diversity we
470 wished to calculate the probability that DME or RME would become common based on
471 both strategies being present at some low initial frequency. We therefore adjusted our
472 calculations to include the chance that both strategies would become established at
473 appreciable frequencies, in which case the strategy with higher geometric mean fitness
474 is expected to prevail. Thus, for parameter values that give DME a higher geometric
475 mean this is simply U_{DME}. However, if RME had a higher geometric mean this
476 becomes $U_{DME}(1 - U_{RME})$.

477 We also performed stochastic simulations of the spread of DME and RME
478 genotypes into an ancestral population composed mostly of genotypes that produce only
479 the normoxia adapted phenotype. We used population size of 1,000 and an initial
480 frequency of both ME strategies of 0.005. RME strategies were assumed to use the
481 optimal frequency of producing normoxia/anoxia adapted phenotypes. Again, we used
482 the Wright-Fisher model to simulate changes in genotype frequency. In each generation
483 the relative number of offspring produced by each genotype was simply the frequency
484 of genotype *i* multiplied by the average fitness of genotype *i* in the current environment.
485 The number of individuals of genotype *i* was updated by sampling from a multinomial
486 distribution where the probability of drawing genotype *i* was the reproductive output of
487 genotype *i* divided by the total reproductive output of the population. We calculated the
488 population level fitnesses as they would have been measured in our experimental assays
489 and used these to calculate the geometric mean fitness of the population.

490 For our second modelling approach we constructed lists of sequences of 12
491 generations of normoxia or anoxia and then used the assayed fitness values to calculate
492 the "expected adaptation" of the derived predictable populations relative to the ancestral
493 population. The total frequency change of a genotype over multiple generations is a
494 simple function of the product of the relative fitness of the two genotypes in each
495 generation. We therefore defined the expected adaptation as $A_s = (\prod_{t=1}^{12} w_t)$, where the
496 relative fitness of the novel genotype is w_t in generation *t*, and A_s measures the per-
497 generation fitness advantage of the novel genotype in an environmental regime
498 characterized by sequence *s*.

499

Acknowledgements

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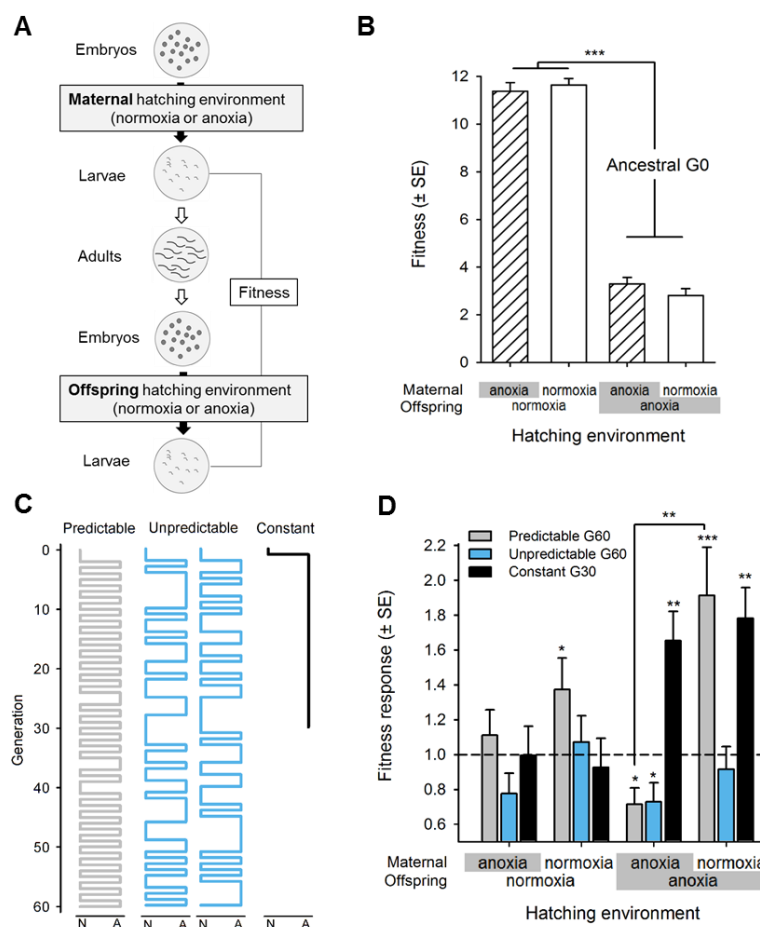601   Genetics 191: 895-906.

Figures

Fig. 1. Experimental evolution in temporally fluctuating environments. (A) Four possible mother-offspring environments were implemented during experimental evolution by varying the maternal and offspring hatching environments between normoxia and anoxia. Fitness is defined as the per capita growth rate measured at the first larval stage over one life-cycle. (B) Fitness of the ancestral population across all four maternal-offspring hatching environments. Offspring survival after anoxia exposure is severely hampered, independently of maternal hatching environment, when compared of the normoxia conditions to which the ancestor was adapted (14). (C) Environmental sequences of normoxia (N) and anoxia (A) imposed for 60 generations. (D) After experimental evolution, fitness of predictable (grey), unpredictable (blue) and constant (black) populations relative to the ancestor population (dashed line), across the four combinations of maternal-offspring hatching environments. Mean and error least square estimates are shown linear mixed effects models LMM (B, D). Significant Student t test fitness responses above each bar and planned post-hoc Tukey t test contrasts among hatching treatments: *P<0.05; **P<0.01; ***P<0.001.

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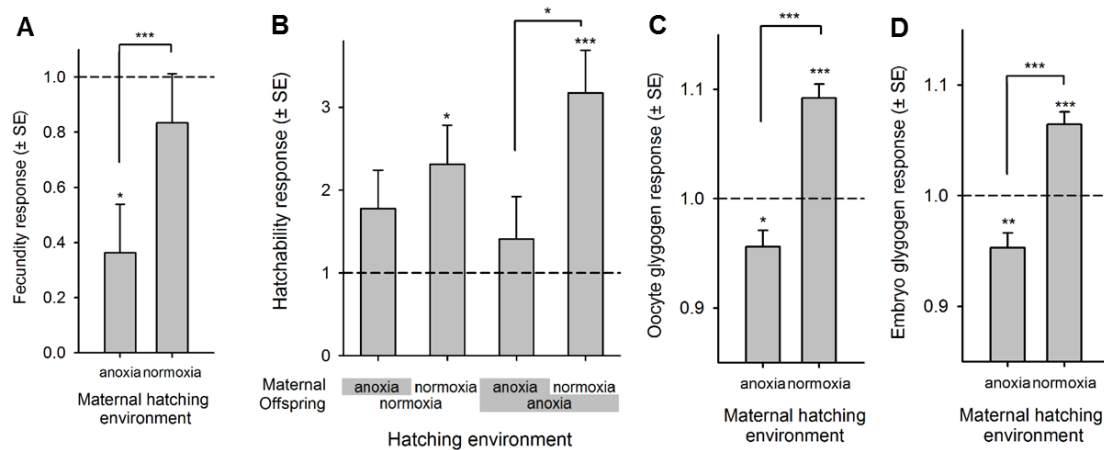
Fig. 2. Experimental evolution of maternal glycogen provisioning protects embryos from anoxia but at the expense of fecundity. Fecundity (A), hatchability (B), oocyte (C) and embryo (D) glycogen content of the predictable populations after experimental evolution relative to the ancestral population (dashed line). For all panels, mean and error least square estimates are shown after LMM, significant evolutionary responses above each bar, and planned Tukey t test contrasts among oxygen level treatments: *P<0.05; **P<0.01; ***P<0.001.
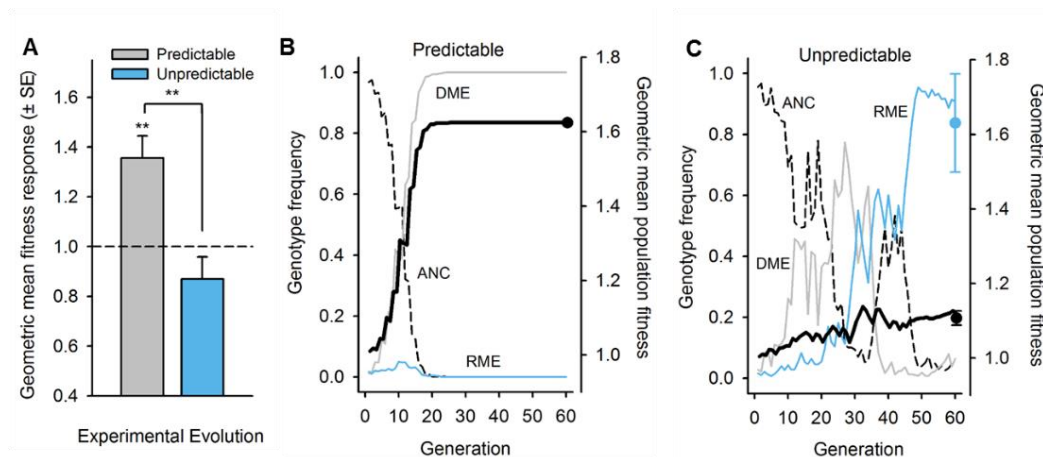
Fig. 3. Maternal bet-hedging does not explain adaptation under irregular anoxia environments. (A) The geometric mean fitness of the oxygen level transitions across generations experienced by the predictable (normoxia-anoxia and anoxia-normoxia) and unpredictable populations (all four pairwise combinations) relative to the ancestral state (dashed line). Mean and error least square estimates are shown after ANOVA, significant evolutionary responses above each bar, and an $F_{1,6}$ test contrast among experimental regimes: **$P<0.01$. (B, C) Simulations mimicking the evolution of maternal effects during experimental evolution (see Methods). (B) The probability of environmental transition was set to 0.95, as the predictable populations faced during experimental evolution. Relative phenotypic fitness values are of $W_{A\text{-to-}N}=0.335$ and $W_{A\text{-to-}A}=2.63$, similar to those of Fig. 1D. The deterministic maternal effect genotype (DME; grey line) reaches fixation at generation 24, during which the population geometric mean fitness (black line) smoothly increased to the expected value of the square root of $2.63 = 1.624$. Fifty simulations with the same parameter values show that at generation 60 there is always fixation of the DME genotype and thus the same population geometric mean fitness (black circle). The randomized maternal effect genotype (RME; blue line) does not invade the population composed by the ancestral genotype that does not express maternal effects (ANC; dashed line). (C) As in panel (B) but with the probability of environmental change at 0.55.
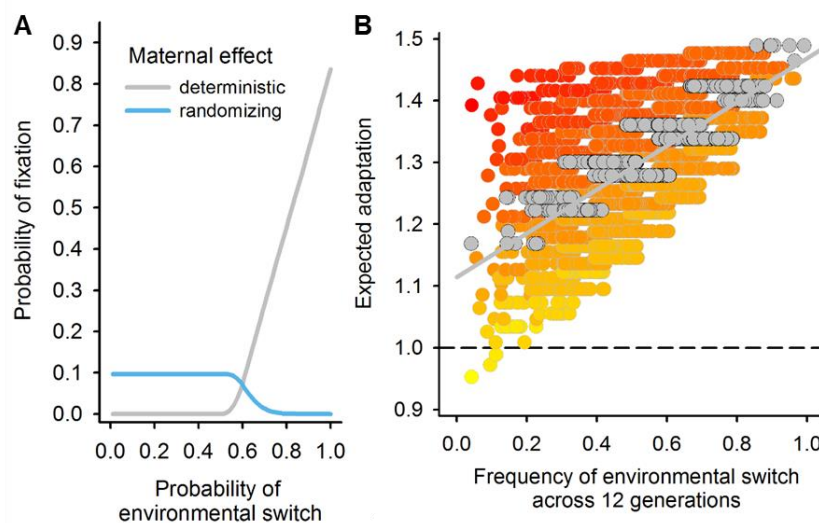
Fig. 4. Maternal deterministic effects and associated fitness benefits can underlie adaptation to fluctuating environments. (A) The probability of fixation of a genotype expressing deterministic maternal effects (gray line) or randomized maternal effects (blue line), when invading the ancestral population without maternal effects. Results are from 10,000 randomly drawn fitness parameters with $\gamma = 2.0$, producing approximately 2-fold fitness effects on average (from Fig. 1D). Other distributions yield qualitatively similar results. Fixation probabilities were calculated with effective population size of 10^3 [32,42], and an initial frequency of 0.01 (see Methods). (B) Expected adaptation of the predictable populations relative to the ancestor population (dashed line) if they were to face fluctuating sequences of normoxia and anoxia for 12 generations. Data is jittered along the x-axis for clarity. Each circle shows one environmental sequence out of the 2^{12} possible, with the yellow-to-red color gradient depicting increasing frequency of normoxia generations, and grey circles and line representing sequences in which normoxia and anoxia generations are equally represented.

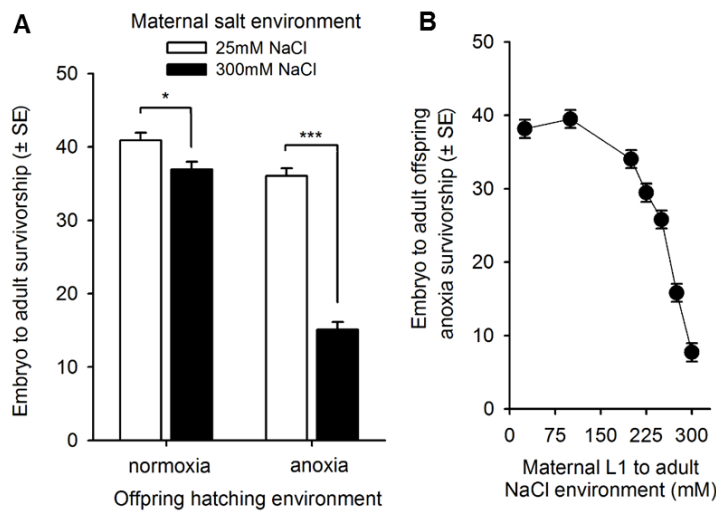663 **Supplementary Figures**

664

666 **Fig. S1. High salt maternal environments impair embryo survival to anoxia.** (A)
667 Embryo to adult survivorship under normoxia and anoxia hatching environments, after
668 maternal rearing from L1 larval stage to adulthood at low (white) or high (black) salt
669 concentrations. Four different genotypes (L126, L142, L188, L244) derived from a lab-
670 adapted population (A6140) were used in these assays (14). L1s from each of the inbred
671 lines were seeded in either 25mM NaCl or 300mM NaCl NGM-lite plates with *E. coli*.
672 After 96h, fifty embryos were hand-picked and transferred to 6cm Petri dishes with
673 25mM NaCl NGM-lite and a 10uL drop of E. coli, and then placed under normoxia or
674 anoxia conditions for 16h (see Materials and Methods). After 96h, the number of adults
675 was scored for survivorship. ANOVA mean and error least square estimates are
676 presented: maternal-offspring treatment: $F_{3,63}$ P<0.001, interaction genotype with
677 maternal-offspring treatment: $F_{9,63}$ P<0.001. Post-hoc Tukey t-tests contrasts among
678 maternal salt treatments: *P<0.05; ***P<0.001. (B) Reaction norm of embryo to adult
679 survivorship over different maternal salt concentration environments for genotype L244
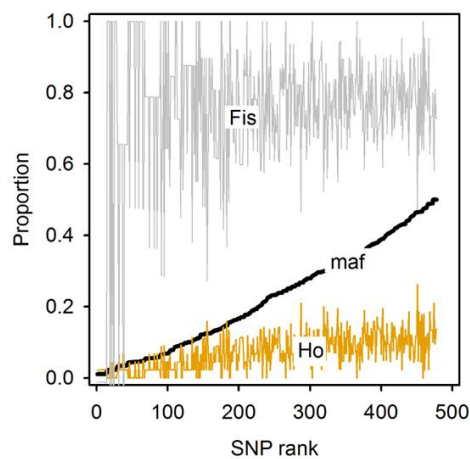680 (n=20 per treatment).

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Fig. S2. Standing genetic diversity in the high-salt adapted ancestor population.
Observed heterozygosity (Ho) and fixation indices (Fis) of 478 SNPs covering the
whole genome and ranked according to increasing minor allele frequency (maf). High
Fis values indicate a high level of inbreeding due to a high proportion of hermaphrodites
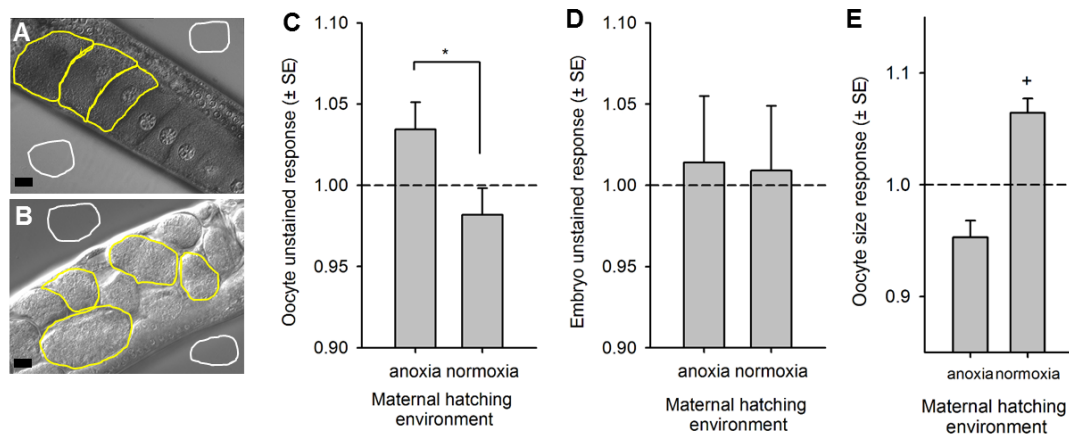reproducing by self-fertilization.

Fig. S3. Iodine staining of glycogen in oocytes and embryos. (A, B) Glycogen content was quantified in iodine vapor stained oocytes and embryos within hermaphrodites (see Materials and Methods). Illustrative photographs of stained oocytes and unstained embryos are shown from ancestral hermaphrodites. The width of the scale bar is 10μm. The ratio of the mean pixel intensity of all the delineated oocytes/embryos in an individual (yellow lines) and the mean pixel intensity of the corresponding agar pad was used for analysis (solid white lines). The ratio values of control unstained oocyte (C) and embryo (D), or oocyte size (E), of the predictable populations at generation 60 relative to the ancestor state (dashed line). After LMM, we did not detect evolutionary responses, although values were higher when hatching under anoxia relative to hatching under normoxia ($t_{24.4}$ P=0.01). Oocyte size in normoxia-hatched hermaphrodites may have increased relative to the ancestor (t_9 P=0.06), but there is no correlation between oocyte size and its pixel intensity ratio in stained hermaphrodites (Pearson's ρ=-0.04, t_{78} P=0.73).
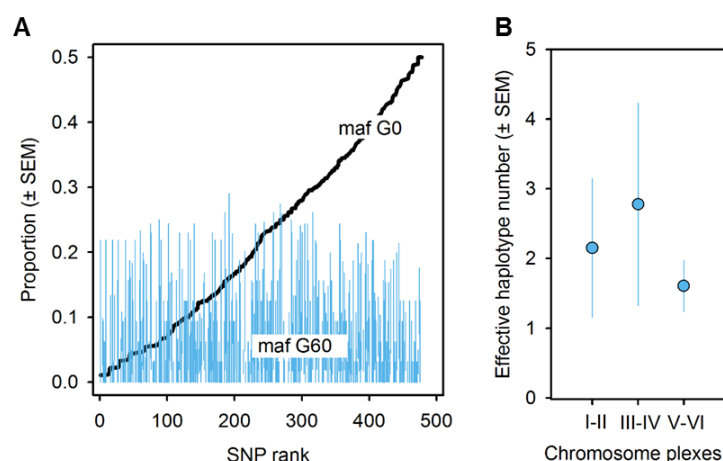
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Fig. S4. Genetic diversity in the unpredictable populations. (A) 478 SNPs are ranked according to increased minor allele frequencies in the ancestor population (from Fig. S2, G0), and compared to the minor allele frequencies of the unpredictable populations at generation 60 (G60). (B) The effective haplotype number found in chromosomes I-II, III-IV, and V-VI (see Methods). Blue bars show the mean and one standard error of the mean. The loss of genetic diversity during experimental evolution implies that only a few genotypes could have been responsible for potential fitness responses due to randomized maternal effects.