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Physiological response to three environmental stressors predicts fitness and genome quality but not base substitution rate in caenorhabditis elegans

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PHYSIOLOGICAL RESPONSE TO THREE ENVIRONMENTAL STRESSORS PREDICTS FITNESS AND GENOME QUALITY BUT NOT BASE SUBSTITUTION RATE IN *CAENORHABDITIS ELEGANS*

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MASTER'S THESIS

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ABSTRACT

Since DNA mutations occur all the time and may have deleterious effects on fitness (survival and reproduction), organisms expend substantial metabolic resources to find and repair mutations. This metabolic expenditure is termed the cost of fidelity. If an organism is in "poor" condition and does not have enough metabolic resources, it may no longer be able to find and repair mutations (Agrawal 2002). In this thesis, we looked at whether physiological condition, measured as survival and development in the presence of an exogenous stress ('robustness'), correlated with mutation rate (experiments 1 and 2) or fitness (experiment 2). We measured physiological robustness of nematodes exposed to three different stressors: high heat, high free radicals, and high salt. The nematodes belonged to two Rhabditid nematode species and either had unmutated genomes (ancestral control) or genomes that had accumulated mutations. In both high heat assays, we found that heat was effective at reducing nematode survival. We found that thermotolerance was higher in the HK104 strain of *Caenorhabditis briggsae* than the N2 strain *C. elegans*. Within the N2 strain, thermotolerance correlated with fitness. In experiments 1 and 2, exposure to high free radicals and high salt reduced nematode survival and slowed nematode development. The N2 strain was significantly more tolerant of free radicals and salt than the HK104 strain. Within the N2 strain, tolerance to free radicals and salt correlated with fitness. No robustness trait significantly correlated with mutation rates in experiment 1; we could not test for correlations with future mutation rates in experiment 2 because mutation rate data are not available at this time. Overall, robustness did not predict base substitution rate but was successful at predicting fitness when fitnesses were very different

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INTRODUCTION

Purpose: The purpose of this research project was to test whether physiological robustness (higher survival and development) of nematodes would be related to base substitution rates, fitness, or mutational declines in fitness.

Mutations are considered the ultimate source of genetic variation and key to evolution by natural selection. Mutations are defined as any changes to the nucleotide sequence of DNA, including point mutations (changing one nucleotide to another), insertions/deletions, or translocations. Mutations can occur due to exogenous (environmental) factors or endogenous (genomic or physiological) factors. Mutations directly result from exogenous mutagens such as UV radiation (e.g., Witkin 1969) and damage from chemicals (e.g., Regen and Setlow 1974), or indirectly from environmental stressors (such as overheating) that impair DNA repair (e.g., Lichtenauer-Kaligis *et al.* 1993). Endogenous sources of mutation include errors that occur during DNA replication (e.g., Lichtenauer-Kaligis *et al.* 1993) and by-products of cellular metabolism that damage DNA (e.g., Hsie *et al.* 1986). The causes of mutations and degree to which mutations are detected and repaired varies extensively across organism type; in the current study, we are focusing on multicelled eukaryotes unless stated otherwise.

Mutations are classified by their effects on fitness, an individual's ability to survive and propagate its genes. Mutations can have one of three effects on fitness: neutral, deleterious, or beneficial. The majority of mutations will have no phenotypic (detectable) effect and thus are considered to be neutral (Kimura 1985). Most mutations fall into this category for two main reasons. First, mutations will have no phenotypic effect because most regions of a chromosome do not code for a gene product nor regulate

expression of other genes (Elgar and Vavouri 2008). Most mutations that occur in these non-coding regions have no phenotypic effect. However, since nucleotides are “read” in groups of three during the translation step of protein synthesis, a frame-shift, or reading frame shift, can occur when an insertion or deletion of nucleotide(s) alters which nucleotides fall into a given reading frame (Farabaugh 1996). Insertion/deletion mutations in noncoding regions can alter reading frames in adjacent coding regions, thus having a phenotypic effect (beneficial or deleterious). Second, the mutations that arise in coding regions may not result in alteration of the amino acid sequence found in a protein. During protein synthesis, nucleotide triplets in the messenger RNA (‘reading frames’) are translated by ribosomes and the corresponding amino acid is added to the growing amino acid chain. Since up to six codon combinations can code for the same amino acid, this redundancy in the coding, particularly at the third nucleotide position, safeguards the amino acid sequence from small changes in the DNA sequence.

Neutral mutations may comprise the majority of mutations occurring in a genome, but it is deleterious and beneficial mutations that ultimately influence fitness (survival and reproduction). Deleterious mutations are defined as changes in the DNA sequence that result in a reduction of fitness. Beneficial mutations result in an increase of fitness. Since harmful mutations will decrease fitness, it is important to find and repair them as quickly as possible. For this reason, most organisms are constantly using enzymes, typically DNA polymerases and a group of DNA repair enzymes, that scan the DNA sequence for damaged nucleotides or mutations and correct any mistakes (Maki 2002). This proofreading is particularly widespread during DNA replication, the largest endogenous source of mutations. In addition to fixing mutations after they occur, cells

will also take measures to prevent mutagenic damage such as suppressing nucleotide mispairing at the terminal end of DNA and synthesizing proteins to wrap around and protect DNA (Maki 2002).

The extensive repair and protective processes ensure that mutations are relatively rare events, a strategy that is generally assumed to be beneficial because the vast majority of mutations with an observable effect are deleterious (Morgan 1903). It is typically assumed that it is in a lineage's best interest to reduce the mutation rate close to zero (Baer *et al.* 2007). In reality, the number of mutations occurring (mutation rate) can never be zero. If the mutation rate were zero, there would be no opportunity for beneficial mutations to occur (e.g. Leigh 1973). Additionally, the metabolic costs of detection and repair are so high that it is unlikely that all mutations are detected/repaired (e.g. Kimura and Ota 1973; Sniegowski *et al.* 2000). There likely exists a trade-off between expending the metabolic energy for surveillance/repair of mutations and allowing mutations to remain and affect fitness. This trade-off is commonly known as the "cost of replication fidelity" (e.g. Kimura 1967; Sniegowski *et al.* 2000).

While it is known that mutation rates vary within and among species (Sturtevant 1937; Drake *et al.* 1998; Baer *et al.* 2007; Haag-Liautard *et al.* 2007), the factors influencing variation of mutation rates are poorly understood. It is possible that variation in the mutation rate is due to optimization at the species and individual level (e.g. Leigh 1973; Kondrashov and Houle 1994). Notably, mutation rate optimization may be subject to environmental conditions (Bjedov *et al.* 2003; Galhardo *et al.* 2007), particularly in cases where environmental factors increase an organism's stress levels, thereby impacting the "cost of replication fidelity." Evidence for this has been found in

prokaryotes exposed to environmental stress; the prokaryotes had reduced fitness and higher expected mutation rates when exposed to stress (Hall 1992; Goho and Bell 2000b). Whether these elevated mutation rates are a consequence of reduced metabolic resources available for mutation detection/repair or are an adaptive response to stress is unclear. The effect of environmental stress on mutation rate optimization led Agrawal (2002) to propose a model of a fitness-dependent mutation rate.

In 2002, Agrawal formulated a theoretical framework for modeling mutation rate as a function of condition in an organism. Empirical evidence in prokaryotes and single cell eukaryotes has shown that physiological condition can affect the mutation rate (e.g. Hall 1992; Goho and Bell 2000a; Galhardo *et al.* 2007), presumably by impacting the cost of replication fidelity. Agrawal (2002) used the results from these studies to model what he termed a "condition-dependent mutation rate." In Agrawal's model, individuals in poor condition will tend to have a higher mutation rate and pass more mutations to their offspring than will individuals in good condition (Agrawal 2002). Condition and mutation rate thus create a feedback loop of declining condition and increasing mutation rate through multiple generations, in theory leading to extinction. Condition-dependent mutation rate was not formally tested in multicellular eukaryotes until 2008 when Agrawal and Wang looked at dietary condition and gamete repair in female *Drosophila melanogaster*. They found that females with good diets were able to repair gametes, while females with poor diets were unable to properly repair gametes. While not conclusive and unspecific as to the mechanisms of condition-dependent mutation rate in this instance, this study lays the framework for all future condition-dependent studies in eukaryotes (Baer 2008a).

While Agrawal set the framework for a condition-dependent mutation rate, it is important to note that Agrawal did not specify exactly the type of condition that would lead to this phenomenon. Our collaborator, Charles Baer of the University of Florida interpreted condition-dependent mutation rate to mean the condition of an organism's genome. Baer is testing the hypothesis that an organism's genomic condition (base substitution rate) could influence mutation rate. As such, Baer has termed the genome condition-dependent mutation rate a 'self-dependent mutation rate' because genome condition and mutation rate are based on the individual at the genomic level (Baer, pers. comm.). On the other hand, Joanna Joyner-Matos, interpreted Agrawal's hypothesis to mean an organism's physiological condition. An organism in better physiological condition will have a lower mutation rate than an organism in poor physiological condition because it is able to divert more energy to the cost of replication fidelity (Joyner-Matos, pers. comm.).

For our experiment, we used Rhabditid nematodes of the genus *Caenorhabditis* to explore the applicability of physiological condition in Agrawal's condition-dependent hypothesis. Nematodes offer an exemplary study system because they are small, readily available, easily cultured on agar plates, have short generation times, and high reproductive output. Certain strains of *Caenorhabditis elegans* have been kept in a lab setting for over fifty years (Brenner 1974). Additionally, *C. elegans* was the first multicellular eukaryote to have its genome completely sequenced (Consortium 1998). The functions of most of the major genes are annotated and well understood. Most *C. elegans* are self-fertilizing hermaphrodites that complete an entire generation every four days. They can be frozen and stored at -80°C, and then thawed, allowing a record to be

kept of each generation. Males can also arise in a population, allowing for outcrossing, but they are easily identified and removed to stop outcrossing. The genus *Caenorhabditis* has several well-studied species, including *C. elegans* and *C. briggsae*; comparisons between congeners are particularly useful for commenting on evolutionary trends in mutation (e.g., Baer *et al.* 2005; Baer 2008b).

Since mutations are relatively rare events, they are hard to study if one must wait for them to occur. By artificially limiting the effects of natural selection while maximizing the occurrence of mutations, we can begin to study mutations in a relatively short time period. One of the best approaches to studying mutations in this manner is the process of mutation accumulation (MA). Mutation accumulation, which is commonly used in Rhabditid nematodes, allows spontaneous mutations to accumulate in the relative absence of natural selection (Lynch *et al.* 1995; Vassilieva and Lynch 1999; Baer *et al.* 2005). MA experiments are started with highly inbred, self-fertilizing, hermaphroditic nematodes that are assumed to be completely homozygotic, meaning they have no genetic variation. Homozygosity is achieved by in-breeding ('selfing') a nematode lineage for up to twelve generations (Baer *et al.* 2006). A MA experiment tracks the occurrence of mutations in independent lineages; each lineage started with a single, inbred nematode and replicate lineages are started with sibling nematodes. Mutations are allowed to accumulate in a lineage by forcing the lineage through successive genetic bottlenecks (Figure 1). A bottleneck occurs when a population is forced to propagate with a smaller gene pool, restricting the genetic variance of the future population to the variance present at the time of the bottleneck. In self-fertilizing *Caenorhabditis* bottlenecks are achieved by moving a single hermaphrodite; any mutations that occurred

in the sperm or egg that made the (randomly) selected hermaphrodite, can be fixed in that particular lineage. Since *Caenorhabditis* complete a generation every four days, it is possible to run a mutation accumulation experiment for hundreds of generations in a relatively short period of time, generating sets of "MA lines" that have unique sets of mutations.

In a typical MA experiment (e.g. Baer *et al.* 2005), the mutation rates are estimated by calculating the difference in fitness, or total reproductive success, in the MA lines in comparison to the unmutated ancestral control. This difference in fitness is typically termed a "mutational decline in fitness" since fitness in the unmutated ancestors and MA lines are measured simultaneously and blind to line identity (ancestor versus MA). Differences in fitness between nematodes from MA lines and from the unmutated ancestor are attributed to deleterious (or beneficial) effects of mutations. Fitness of MA lines in nematodes tends to be 10 – 20 percent lower than that of the unmutated ancestor (Figure 2) (Baer *et al.* 2005). In the N2 strain of *C. elegans*, 200 generations of MA significantly decreased fitness, from 220.1 ± 8.4 (mean ± SEM) offspring in the unmutated ancestor to an average of 178.3 ± 9.3 offspring across 67 MA lines (Baer *et al.* 2005). The 200 generations of MA had an even greater effect on the HK104 strain of *C. briggsae*, with unmutated ancestors having 99.6 ± 13 offspring but the 67 MA lines averaging 37 ± 20 offspring (Baer *et al.* 2005). Additionally, variance in fitness tends to be higher across MA lines than across replicates of the unmutated ancestors; variance in fitness (V_b in Table 1, Baer *et al.* 2005) increased by 11.5% per generation of MA in the N2 strain and 2.39% per generation in the HK104 strain. Mutation rate (U_{MIN} in Table 1, Baer *et al.* 2005) is estimated by dividing the per-generation (of MA) change in mean

fitness by the per generation increase in among-line variation (Bateman 1959; Mukai 1964). The mutation rate estimate for N2 was 0.0083 ± 0.007 and the mutation rate estimate for HK104 was 0.074 ± 0.04 (Baer *et al.* 2005). Mutational declines in fitness thus indicate estimated mutation rate, such that MA lines with the lowest fitness are estimated to have experienced the greatest number of mutations, or have the highest mutation rate.

For our study, we worked with two strains of nematodes that underwent mutation accumulation (N2 strain of *C. elegans* and the HK104 strain of *C. briggsae*; Baer *et al.* 2005). We conducted assays on the unmutated ancestors (generation 0 controls) and select MA lines from each strain. *Caenorhabditis briggsae* is a sister species to *C. elegans*, but for reasons not well understood, the HK104 strain of *C. briggsae* tends to accumulate more mutations during MA and is more susceptible to extinction during MA (e.g. Baer *et al.* 2005; Phillips *et al.* 2009). In comparison to *C. elegans*, *C. briggsae* nematodes tend to have 'lower' fitness and 'poorer' physiological condition (Joyner-Matos *et al.* 2009) and a comparably small body size following MA than do nematodes from the *C. elegans* strains under similar conditions (Ostrow *et al.* 2007).

We conducted two separate experiments to test whether poor physiological condition correlates with mutation rate and fitness (as documented by Baer *et al.* 2005; Denver *et al.* 2009). Both of the experiments took advantage of our collaboration with the Baer lab and incorporated strains used in on-going Baer lab experiments. We were restricted to correlational studies, as opposed to an MA experiment, which could explore causative relationships between condition, mutation rate, and fitness, because MA experiments tend to require four years to complete.

In experiment 1, we worked with several Baer MA lines (Baer *et al.* 2005) from the *C. briggsae* strain HK104 and the *C. elegans* strain N2. Randomly selected subsets of the Baer MA lines have been sequenced as collaboration between Baer and Dee Denver at Oregon State University. The results from the whole-genome sequencing of the select N2 MA lines has been published (Denver *et al.* 2009); the results from the HK104 sequencing are in the final review stage of publication, but we have access to them through Denver (Denver *et al.* 2012, in review). The goals of this sequencing project were to get exact counts of mutations present, test whether estimated and actual mutations rates correlated, and characterize the spectra of mutations in the different MA lines. Since we now have exact counts of mutations in these selected N2 and HK104 MA lines, we have been able to test whether physiological condition can be used to predict base substitution rate. It is important to know that when we refer to exact mutations, we are referring to the base substitution rate, but we are not including insertion/deletions or genome rearrangements. This was done to compare results from experiment 2 where condition is used to predict mutation rate. Since we know the genomic condition of the lines after the sequencing, we can test whether the effects of physiological condition predict base substitution rate.

In experiment 2, we explored the physiology of ten MA lines from Baer's experiment on self-dependent mutation rate. To test whether mutation rate can be self-dependent, Baer selected ten of his 100 N2 strain (*C. elegans*) MA lines from his 250-generation MA experiment (Baer *et al.* 2005), and put these ten MA lines through an additional 125 generations of MA, or a "second-order MA" (Baer, pers. comm.). Five of the selected lines had the highest fitness (estimated to have the lowest base substitution

rate) of the 100 original N2 MA lines and five of the selected lines had the lowest fitness (estimated highest base substitution rate). Baer hypothesized that if base substitution rate (genome condition) influences mutation rate, then the 'high fitness' MA lines will have a lower mutation rate in the second-order MA experiment than will the 'low fitness' MA lines. The Baer lab has completed the 125 generation second-order MA and the fitness assays on these ten lines at generation 0, generation 250 (1^{st} order), and generation 375 (2^{nd} order). Baer has completed the analysis of the data and the publication is currently in preparation (Baer, pers. comm.). The preliminary analysis of his results reveals that mutational declines in fitness were greater in the low fitness MA lines than in the high fitness MA lines. In the second-order MA (MA generations 250 – 375), fitness of the low fitness MA lines decreased by 1.81 ± 0.4% per generation while fitness of the high fitness MA lines decreased by 0.89 ± 0.23% per generation (Baer, pers. comm.). One low fitness MA line, MA line 579, had somewhat aberrant results in the fitness assay and (as of April, 2012) is being re-assayed. When MA line 579 is removed from the data set, fitness of the low fitness MA lines decreased by 2.13 ± 0.32% per generation, which is a significantly greater decline than that detected in high fitness MA lines (Baer, pers. comm.).

The Baer lab was gracious enough to send us the second-order ancestral lines and the original unmutated (generation 0) ancestor for our experiments. We used the second-order MA lines as a means to forecast whether 'future' mutation rate can be predicted based on physiological condition. While our study looked solely at physiological condition, it is important to note that the influence of genomic condition cannot be disentangled from physiological condition. The genomic condition will always have

underlying influence simply because genes strongly influence an organisms' physiology and therefore its fitness.

By comparing the results of experiment 1 and 2, the end product will have more explanatory power than the individual experiments. Experiment 1 compares species and allows us to test whether base substitution rate in MA nematodes correlates with physiological condition. Experiment 2 is similar to experiment 1, except that it compares whether we can predict future mutation rate based on physiological condition of MA experiment ancestors. Taken together, these studies use a correlational approach to explore how fitness, mutation rate, and physiological condition are related. We assessed physiological condition by measuring resistance to multiple environmental stressors.

Stress assays typically consist of placing nematodes in a stressful environment and measuring their "resistance" to the stress, typically through either development to adulthood or survival (e.g., Lithgow *et al.* 1994). We based our stress assays procedures on work done by the Joyner-Matos lab. Undergraduate students in the Joyner-Matos lab assayed the heat stress tolerance of the sequenced N2 MA lines (Denver *et al.* 2004) during the first week of July 2010. They found that the number of mutations per MA line was significantly and negatively correlated with heat tolerance (data not shown). We repeated the heat assay procedure on the sequenced N2 and HK104 MA lines (experiment 1) and the second-order MA ancestors (experiment 2) to test whether heat tolerance correlated with future base substitution rate (experiment 1) or mutation rate (experiment 2).

Our second method of testing sensitivity to an environmental stress, we exposed nematodes to paraquat. Paraquat (N,N'-dimethyl-4,4'-bipyridinium dichloride, also

known as methyl viologen) is a chemical that was used at one time as a pesticide and herbicide in the United States. Paraquat causes singlet oxygen radical formation in exposed tissues (Fujii *et al.* 2005). The free radicals may interact with a cell's DNA and cause DNA damage that leads to mutations. If the paraquat levels are high enough, they can kill an organism. Free radical resistance appears to be linked to multiple genes that are involved with senescence and behavior, both of which are altered by mutation accumulation (Joyner-Matos *et al.* 2009; Busuttil *et al.* 2003; Yanase *et al.* 2002).

Our third stress assay explored tolerance to hyperosmotic stress, or elevated salt (sodium chloride) levels. We optimized the salt levels using a spectrum of salt concentrations with the control strain (N2 ancestor). With the salt assay, we tested whether correlations exist between salinity tolerance and base substitution rate (experiment 1) and mutation rate (experiment 2).

While the three assays described above are fairly well-established in nematode biology (see references above), the collected data tend to be survival and/or development, but metrics for overall "activity" or condition are not well-described. We are interested in quantifying not just whether the nematodes survive the stress assays and, in the case of the paraquat assay, survive and develop to adulthood. To this end, we developed an assay to measure activity levels of nematodes when exposed to stressors. Most "activity" assays, or behavioral assays, for nematodes involve a nematode's ability to chemotax, or sense a chemical (attractant or repellant), and move towards or away from the chemical. Chemotaxis is well-studied from the neurobiological angle (e.g. Bargmann 2006) and has been shown to be a fitness component (declines in MA lines; Ajie *et al.* 2005). However, chemotaxis assays are conducted under normal conditions and may not be appropriate to

use with the added component of an environmental stressor. We therefore conducted a 'behavioral' analysis of motility (Solomon *et al.* 2004), or how active the nematodes are in moving over the (high salt or paraquat-containing) surface during a period of time.

Hypotheses

For experiment 1, we hypothesized that the physiological robustness (higher survival and/or development) of the sequence N2 and HK104 lines would be inversely related to base substitution rate (Denver *et al.* 2009; Denver *et al.* 2012, in review). Those lines identified by Denver as having the highest base substitution rate would have the lowest survival and/or development. We also predicted that we would detect differences in physiological robustness between strains. For example, *C. briggsae* tends to be more tolerant of higher temperature than *C. elegans* (Matsuba *et al.* 2012, in review).

For experiment 2, we hypothesized that the MA lines identified by Baer as 'high fitness,' (those that he predicts will have low mutation rates in his second-order MA experiment) would be more physiologically robust than will the 'low fitness' MA lines. Physiological robustness means higher survival and/or development and greater motility levels during exposure to high temperature (no motility assay), high salt, or paraquat. This hypothesis groups the ten second-order MA lines into high- and low-fitness groups with 5 MA lines each (as defined by Baer). We also hypothesized that our measures of physiological robustness (survival, development, and motility) would directly correlate with the mutational declines in fitness measurements (inversely related to the estimated mutation rates) that occur during the second-order MA (MA generations 250 – 375; C. F. Baer, in revision).

MATERIALS AND METHODS

To address the objective, "a correlational approach to explore how fitness, mutation rate, and physiological condition are related" we conducted two experiments that were used to generate estimates of fitness and mutation rate.

Experiment 1 used the sequenced HK104 (*C. briggsae*) MA lines and sequenced N2 (*C. elegans*) MA lines from the Denver and Baer labs; experiment 2 used the ten second-order N2 MA lines from the Baer lab. All assays were optimized using the N2 ancestor strain for control. The strains and lines arrived cryopreserved in a glycerol/salt solution on ice and were stored in a -80°C freezer.

To begin the experiments, we removed a single cryotube per strain (N2 ancestor, second-order MA ancestor, or sequenced N2 or HK104 MA line) and allowed it to thaw for 15 minutes at room temperature. Upon thawing, any liquid/nematodes in the tube were pipetted onto a small (60 X 15 mm) agar plate. We made the nematode growth medium (NGM) agar plates in 1 L volumes, each plate had approximately 12 mL of the 1 L mix. The 1 L mix had 3.0 g NaCl, 2.5 g peptone, 17 g agar, all in 975 ml DI H_2O and was supplemented after autoclaving with 1 mL of a 1 M $MgSO_4$, 1 mL of a 0.5 M $CaCl_2$, 1 mL cholesterol solution, and 25 mL of "salts solution" that was 1 M KH_2PO_4 and 1 M K_2HPO_4. Agar plates were seeded with the OP50 strain of *Escherichia coli* as a food source. The nematodes were allowed to grow for four days at 20°C and then a single L4 hermaphrodite (larval stage just prior to reproductive maturity) was picked and placed on a new plate to grow at 20°C. This bottlenecking, or selfing, procedure was repeated three times for each strain to remove any variation caused by differences in freezing techniques (removes maternal and grandmaternal effects). At the time of the first bottleneck, we

created five replicates (picked five nematodes) for each MA line and five pseudolines for each ancestral control genotype (N2 or HK104). Every replicate or pseudoline went through the additional two bottlenecks independently to allow us to estimate the variance within MA line or ancestral genotype. After bottleneck generation three, condition assays were conducted on either adult nematodes (heat and behavioral assays) or eggs (salt and paraquat assays). Adult nematodes are those that have progressed beyond (molted) the L4, or larval stage 4, which is easily identified by the presence of the half-moon shaped light patch, which is where the reproductive structures are developing (www.nematodeatlas.org). Young adults no longer have the half-moon shape but do not have visible eggs in their reproductive system. The transition from L4 to gravid (egg-containing) adult typically takes 18 hours (www.nematodeatlas.org).

To conduct the heat assay, we placed adult nematodes on agar plates, incubated them at 35°C, and monitored survivorship. We began each assay by pre-warming small agar plates to 35°C. We then placed 10 adult (reproductively mature and gravid) nematodes on the prewarmed plate and then put them in an incubator set at 35°C, where we monitored them at intervals of two hours. Room temperature control plates were run alongside the hot plates in an incubator set at 20°C. We continued monitoring every 2 hours for up to 40 hours or until all of the adults on a given plate died. We assessed survival as the proportion of adult nematodes surviving at each time interval (Lithgow *et al.* 1994). For both experiments 1 and 2, MA lines were expanded into five replicates per MA line (e.g., MA line 504 had replicates 504A – 504E). Each MA line replicate was assayed on two hot plates (e.g., 504A-1H, 504A-2H; H indicates hot) and one control plate (e.g., 504A-1N; N indicates normal, or control plate), with 10 adult nematodes per

plate. For both experiments 1 and 2, ancestral control genotypes, N2 and HK104, were expanded into five pseudolines (e.g., N2-1 – N2-5); each ancestral pseudoline was assayed on two hot plates (e.g., N2-1-1H, N2-1-2H) and one control plate (e.g., N2-1-1N).

To conduct the paraquat assay, we placed eggs on small agar plates containing 0.5 mM paraquat (N,N'-dimethyl-4,4'-bipyridinium dichloride). We began each paraquat assay by allowing small agar plates with paraquat and normal agar to warm to room temperature (Yanase *et al.* 2002). Then we placed 10 eggs onto a paraquat-containing plate and monitored the plates every twenty-four hours for up to seven days or when individuals on a plate reached adulthood. We counted the number of nematodes present each day and noted the life cycle stage of the living individuals. The assay for a given plate was stopped when individuals on that plate reached adulthood. For both experiments 1 and 2, MA lines were expanded into five replicates per MA line (e.g., MA line 504 had replicates 504A – 504E). Each MA line replicate was assayed on two paraquat plates (e.g., 504A-1P, 504A-2P; P indicates paraquat) and one control plate (e.g., 504A-1N; N indicates normal, or control plate), with 10 eggs per plates. For both experiments 1 and 2, ancestral control genotypes, N2 and HK104, were expanded into five pseudolines (e.g., N2-1 – N2-5); each ancestral pseudoline was assayed on two paraquat plates (e.g., N2-1-1P, N2-1-2P) and one control plate (e.g., N2-1-1N).

We conducted the salt stress assay in 24-well microwell plates, filled with agar that had elevated salt concentrations. Microwells contained 3 ml of NGM agar and were seeded with *E. coli* to ensure the nematodes did not starve. Salt concentrations for 'normal' agar plates are 3 grams of NaCl per liter agar solution (approximately 50 mM),

the elevated salt concentration we optimized to work best with the control strains was 17 grams per liter of agar (290 mM). This salt concentration was lower than those of Solomon et al. (2004), who tested the effects of 500 mM salt; preliminary studies we conducted showed that the MA nematodes die almost instantly at 500 mM salt and therefore we decreased the dose to the one listed above. In a salt assay, we placed five (MA) or ten (ancestor) eggs into wells, and monitored the nematodes every 24 hours for up to nine days or when individuals in a well reached adulthood (Solomon *et al.* 2004). We counted the number of nematodes present each day and denoted the current life cycle stage of living individuals. The assay for a given well was stopped when individuals in that well reached adulthood. For both experiments 1 and 2, MA lines were expanded into five replicates per MA line (e.g., MA line 504 had replicates 504A – 504E). Each MA line replicated was assayed on four salt microwells with 5 eggs per well (e.g., 504A-1S, 504A-2S; S indicates salt) and one control well (e.g., 504A-1N; N indicates normal, or control plate), with 10 eggs per well. For both experiments 1 and 2, ancestral control genotypes, N2 and HK104, were expanded into five pseudolines (e.g., N2-1 – N2-5); each ancestral pseudoline was assayed on four salt microwells with 5 eggs per well (e.g., N2-1-1S, N2-1-2S) and one control well (e.g., N2-1-1N), with 10 eggs per well.

Behavioral assays were conducted only on the second-order lines following the salt and paraquat assays but were not conducted for the heat assay because we cannot run our microscopes in the heated incubator. For a given assay, single, reproductively mature adults from each replicate were picked and placed on a salt, paraquat, or normal (control NGM agar) small agar plates. We recorded data on one nematode per replicate (5 nematodes per MA Line). The plates were placed under a Motic 150 digiscope and

recorded for 10 seconds every 12 hours. We continued to film adults every 12 hours for 5 days, or until it became impossible to distinguish the adult from the progeny. At the conclusion of the filming periods, the videos were imported into ImageJ. ImageJ allowed us to measure the size of the adults and distance traveled (motility). To calculate body size, we used the free drawing tool in ImageJ to draw an outline around the nematode and then ImageJ calculated the area within the outlined shape. To calculate motility, a grid with boxes that were 17.3 pixels per side (300 pixels total) was overlaid on each image. The total number of squares contacted by the nematode's head during the 10 second video indicates the total surface area traveled by the nematode, our metric of motility.

Following the stress assays, we calculated survivorship, proportion alive, development, and population composition for the entire stress assay and at each individual day. For the paraquat and salt assays, we created a metric to simultaneously assess survival and developmental stage. We calculated this "development score" by assigning numerical values to each life stage (L1 stage = 1, L2 stage = 2, L3 stage = 3, L4 stage = 4, adult = 5) and multiplying by the number of nematodes. For example, a plate with 10 live nematodes, all of which are L4 stage, would have a development score of 40. The maximum possible development score was 50 (10 nematodes alive, all adults); development scores less than 50 indicate decreased survival and/or slower development. Where the data were proportions, we analyzed the arc sine square root transformed data.

Before we tested whether the measures of stress resistance correlate with base substitution rate estimates or future mutation rates, we first tested whether we can detect significant among-group or among-line variance. In most MA experiments (e.g., Baer *et al.* 2005), significant among-line variation is detected in the MA treatment but not in the

AC treatment; this increase in variance in the trait in MA lines is generally (but not always) detected in experiments conducted on MA lines. We tested for the presence of significant among-line variation in the sequenced N2 assay and the sequenced HK104 assay, but were not able to do so in the second-order MA experiment since the effect of block (among-block variation) tended to so large that we were unable to detect among-line variation. In the sequenced N2 and HK104 assays, since we conducted all assays with five replicates per MA line, we used the Proc Mixed analysis (general linear mixed model) from SAS (SAS Institute v. 9.2) to test whether differences between MA lines (among-line component of variance) for any given stress assay were significant given the amount of variance among the five replicates within an MA line (within-line component of variance) (Fry 2004). The Proc Mixed procedure partitions the (co) variance in a data set given the model, y=treatment + line + rep + plate + error, with y being our measure of stress resistance and treatment indicating MA line versus ancestral (unmutated) control for any given strain and error representing the within-line variance. Treatment (MA versus ancestor) is the fixed effect, the random effects are line (individual MA line) and replicate (five replicates per MA line) and plate (up to four per MA line); with line randomly nested within treatment, replicates present for each line, and plate present for each replicate. This procedure provides p-values indicating whether estimates of among-line variance are significantly different from zero (give the amount of variance within lines). Variance components are estimated by restricted maximum likelihood and trait means are estimated by least squares.

We used several statistical procedures to test hypotheses concerning the effects of stress, the differences between MA treatments (ancestor versus MA) and the correlations

with mutational properties. For both experiments, we analyzed the same metrics of survival and development, which are defined in Table 1. To test whether the stress treatments were effective, we analyzed one metric of survival (a different metric for each type of stress assay, see appropriate sections in Results) and one metric of development (for most salt and paraquat assays). Data were pooled within a stress treatment (e.g., heat stress for 18 hours) and across MA treatment (MA or AC), and across MA line or fitness category. We used paired T-tests, or nonparametric sign rank tests (for dependent samples), to test whether survival or development differed between nematodes exposed to stress conditions and those in control conditions using alpha error rates corrected by the total number of comparisons (Bonferroni correction).

In experiment 1, we next tested whether various metrics of survival and development varied between ancestors and MA within each strain using the Proc Mixed procedure as outlined above. This procedure, a type 3 test of fixed effects, tests whether the trait varies significantly between AC nematodes and MA nematodes, given how the variance is partitioned into MA treatment, Line, Replicate, and Plate. This type 3 test of fixed effects was conducted for each trait within each strain individually. Since the analysis is at the level of MA treatment (AC versus MA), the analysis was repeated with slightly different code to generate least square estimates of line means for each trait; these line mean estimates were generated without the Plate information, as the model failed to converge when the Plate term was present. As will be presented in detail in the Results section, few of the type 3 tests of fixed effects were significant and none would be considered significant if we corrected the alpha error rates by the total number of comparisons. We therefore present the p-values for the type 3 tests in the Results section

and interpret them conservatively. SAS code for an example trait for the type 3 test of fixed effects and the slightly modified code that provided least square mean estimates of each MA line are in Appendix 2.

Finally, in experiment 1 we tested whether each measure of physiological condition correlated with the base substitution rates of the sequenced N2 and HK104 MA lines (Denver *et al.* 2009; Denver *et al.* 2012, in review). Since the first (2009) estimate of N2 base substitution rates did not provide error estimates for each MA line (only one genotype was sequenced per MA line), and since Denver *et al.* (2012) did not detect significant differences between the base substitution rates of MA lines in the 2012 paper, we tested whether metrics of stress susceptibility correlated (rather than covaried) with base substitution rates. These correlation analyses were conducted within each strain, on the least square estimates of line means for each set of traits within and across stress assays.

In experiment 2, we first tested whether the stress treatments were effective in altering survival and/or development or behavioral traits in the nematodes from the AC lines and from the five 'high fitness' and five 'low fitness' second-order MA line sets. As described above, data within any stress metric (e.g., survival to 20 hours on heat control conditions) was pooled across fitness category prior to being analyzed by paired t-tests. Alpha error rates were corrected by the total number of comparisons. Since the effect of block was so large (see Results), we could not conduct the Proc Mixed procedure and partition variance into among-line and within-line or even among-fitness category and within-fitness category variance. Instead, to test whether each measure of stress susceptibility differed among fitness categories (AC, high fitness MA, low fitness MA),

we analyzed the data using two-factor ANOVA, with fitness category as one factor and block as the second factor, followed by post hoc tests where appropriate. In the few cases where the block effect and fitness x block interaction terms were not significant, we pooled the data across block and tested whether the trait varied significantly among fitness categories using one-way ANOVA. For each trait, we confirmed that the data were normally distributed and that the variances were homogeneous. In cases where Levene's test for homogeneity of variance failed ($p < 0.05$) or the data were non-normal, we tested for differences between fitness categories using the Kruskal-Wallis test.

To characterize the degree to which the high and low fitness lines differed from the ancestral control, we calculated delta M (ΔM) values for most traits. The ΔM calculates the proportional difference in a trait between the MA line(s) and the ancestral control. When the proportional difference between MA and AC is divided by the number of generations of MA, this value represents an estimate of the per-generation change in trait mean. We do not make the assumption that the ΔM represents a linear change in trait mean since none of the intermediate generations were measured. The ΔM is calculated as: $(Mean_{MA} - Mean_{AC})/(Mean_{AC}*250)$ and is multiplied by 10^3 to help the reader interpret the value. For most traits, ΔM values tend to be negative, indicating that the trait mean (e.g., fitness, body size) is smaller in the MA lines than in the AC; more negative values indicate greater per-generation changes in trait mean, or greater mutational decline in the trait.

We then tested whether each measure of physiological condition correlates with the fitness data and with the estimated mutation rates calculated by Baer and colleagues (manuscript in prep.). We present here preliminary correlation analyses between line

mean estimates for stress traits and line means of mutational declines in fitness in the second order MA lines. We cannot conduct formal covariance tests between our stress assays and the Baer lab fitness data because the final set of second order MA fitness assays are not yet analyzed (one line has been re-assayed). The trends reported here, therefore, do not reflect covariance between stress susceptibility and mutational declines in fitness.

RESULTS

A. Effectiveness of Stress Treatments – Experiment 1

Across all three assay types, nematodes in stress treatments had lower survival and/or slower development than did nematodes in the respective control treatments. These patterns are consistent across MA treatments (MA versus ancestor), MA lines, and strains. Data summaries presented in this section are averaged across MA line and across MA treatment and are not presented in figures or tables. These data will be described in greater detail in subsequent sections.

The most informative measures for the effectiveness of the stress treatments on nematode survival was the survival in heat stress at 22 h (for N2) and 29 h (for HK104) and survival to the day that adults appeared on NGM plates in the paraquat and salt assays. Survival was significantly lower in N2 nematodes exposed to heat stress than in nematodes in control conditions, as indicated at the 22 hour period (proportion alive in control conditions, 0.97 ± 0.04 [average ± SEM]; in heat stress, 0.40 ± 0.23; control vs. heat, $p < 0.0001$). Similarly, survival of HK104 nematodes exposed to heat stress for 29 or more hours was significantly lower than those control conditions (proportion alive in control conditions, 0.9 ± 0.09; in heat stress, 0.56 ± 0.24; control vs. heat, $p < 0.0001$). In

the paraquat assay, survival to day 4, the day at which adults were present on the NGM (control) plates, was significantly lower in paraquat-exposed N2 nematodes than in N2 nematodes on NGM plates (proportion alive on NGM plates, 0.85 ± 0.06; on paraquat plates, 0.60 ± 0.13; NGM vs. paraquat, $p < 0.0001$). This significant effect of paraquat on development was not detected at this time point in HK104 (proportion alive on NGM plates, 0.55 ± 0.07; on paraquat plates, 0.57 ± 0.11; NGM vs. paraquat, $p > 0.05$). In the salt assay, survival to day 3, the day at which adults were present on NGM, was significantly lower in nematodes on salt plates than those on NGM plates, in both N2 (proportion alive on NGM plates, 0.80 ± 0.15; on salt plates, 0.46 ± 0.09; NGM vs. salt, $p < 0.0001$) and HK104 (proportion alive on NGM plates, 0.40 ± 0.18; on salt plates, 0.21 ± 0.15; NGM vs. salt, $p < 0.05$).

The most informative indicator of the developmental consequences of stress exposure was the number of days necessary to develop to adulthood. Nematodes from both strains needed significantly more days to develop to adulthood on paraquat plates than on NGM plates (N2: number of days on NGM plates, 4.0 ± 0, on paraquat plates, 6.0 ± 0.52, NGM vs. paraquat, $p < 0.0001$; HK104: number of days on NGM plates, 3.75 ± 0.10, on paraquat plates, 4.7 ± 3.2, NGM vs. paraquat, $p < 0.0001$). Development also was slowed during salt exposure, with nematodes from both strains requiring more days to reach adulthood on salt plates (N2: number of days on NGM plates, 3.0 ± 0, on salt plates, 5.29 ± 0.34, NGM vs. salt, $p < 0.0001$; HK104: number of days on NGM plates, 3.0 ± 0, on salt plates, 6.61 ± 0.43, NGM vs. salt, $p < 0.0001$).

B. Heat Assay – Experiment 1

The heat stress assays for experiment 1 were conducted in two blocks, the N2 sequenced lines were in block 1 and the HK104 sequenced lines were in block 2. In both assays, young adults were placed on NGM agar plates pre-heated to 35°C and 20°C, and survival was monitored for up to 40 hours. We did not detect any among-line variation in N2 or HK104 AC for any trait related to heat stress. While we did detect among-line variation for some traits in the N2 and HK104 MA lines, the variance was never significantly different from zero and therefore is not presented here.

In the N2 assay, the 22 hour check was also the only time point at which both the 35°C heat stress and 20°C control plates were checked. After 22 hours in control conditions, the proportions of MA and ancestral nematodes that were alive were indistinguishable (MA vs. AC, $p = 0.7557$; 22 H Control on Figure 3). After 22 hours of heat stress, a significantly higher proportion of MA nematodes than AC nematodes were alive (MA vs. AC, $p = 0.0033$; 22 H Heat on Figure 3). Similarly, a significantly higher proportion of MA nematodes survived to 19 and 27 hours than did AC nematodes (19 hours: MA vs. AC, $p = 0.0017$, 19 H Heat on Figure 3; 27 hours: MA vs. AC, $p = 0.0043$, 27 H Heat on Figure 3). While MA line 553 had the highest proportion of nematodes alive at all three time points, MA line 545 had the lowest proportions at all three time points. Means and standard errors of proportions alive are presented in Table 2.

In the HK104 assay, there were no significant differences at 18 hours between the proportions of AC and MA nematodes alive within stress category and between stress treatments (all $p > 0.05$; 18 H Control and 18 H Heat on Figure 4). While there were no significant differences between the proportions of MA and AC nematodes that were alive

at later time points (all $p > 0.05$), AC nematodes tended to have higher survival than did MA nematodes. In general, MA line 263 had the lowest proportion of nematodes alive at each time point; the rest of the MA lines and AC were indistinguishable. Means and standard errors of proportions alive are presented in Table 2.

Since the N2 ancestor also was assayed in block 2, we can compare the performance of *C. elegans* and *C. briggsae* when exposed to heat stress. All of the N2 AC nematodes died prior to 18 hours while at least some nematodes from all of the HK104 lines survived to 40 hours. Within the MA lines checked at 22 hours in both blocks, the range of proportion alive for the HK104 lines was much smaller (HK104 range of survival, 0.82 – 0.94; See Table 2) than the range of proportion alive in the N2 lines (N2 range of survival, 0.07 – 0.73; See Table 2).

C. Paraquat Assay – Experiment 1

The paraquat stress assays for experiment 1 were conducted in two blocks, the N2 sequenced lines were assayed in block 1 and the HK104 sequenced lines in block 2. Eggs from each AC and MA line were placed on agar plates containing paraquat or without paraquat (NGM, control conditions); development and survival were monitored for up to eight days. We did not detect any among-line variation in N2 or HK104 AC for any trait related to paraquat stress. While we did detect among-line variation for some traits in the N2 and HK104 MA lines, the variance was never significantly different from zero and therefore is not presented here. Patterns in survival only reflect survival of hatched nematodes. Hatching success was not analyzed because we cannot determine whether eggs did not hatch due to stress treatment or because they were infertile eggs (some MA lines are more prone to laying infertile eggs; Baer, pers. comm.). For example, in Figure

5, the proportion survived on day 1 (1.0 for MA and AC) indicates that 10 eggs were placed on each plate, it does not indicate that 10 nematodes hatched.

As summarized in Section A, N2 nematodes exposed to paraquat had significantly lower survival than did nematodes on NGM plates. AC and MA nematode survival did not change over time on NGM plates (Table 3). The average proportion of nematodes surviving on paraquat plates decreased over time in both MA and AC lines (Figure 5). Since most nematodes on NGM plates were adults on day 4, we assessed survival at day 4 (Table 3). While MA nematodes tended to have higher survival than AC nematodes on both NGM and paraquat plates, these MA treatment differences were not significant (NGM: MA vs. AC, $p = 0.7075$, Table 3; paraquat: MA vs. AC, $p = 0.2442$, Figure 5 and Table 3). Across all time points, MA line 553 had the highest survival on paraquat and MA line 523 had the lowest survival (Table 3).

As summarized in Section A, N2 nematodes exposed to paraquat had significantly slower development than did nematodes on NGM plates. Nearly all nematodes reached adulthood on day 4 on NGM plates; across MA and AC lines, the proportion of nematodes that were adults on day 4 ranged from 0.8 – 1.0 (data not shown). Nematodes on paraquat plates, on average, needed an additional two days to reach adulthood. The number of days to the first adult appearing on paraquat plates was higher for MA nematodes than AC nematodes (MA, 6.03 ± 0.2 days, AC: 5.5 ± 0.2 days, MA vs. AC, $p = 0.1005$; data not shown). On day 5, the first day any adult nematodes were found on paraquat plates, the proportion of nematodes within an MA or AC line that were adults ranged from 0 – 0.41. The proportion of nematodes that were adults on day 5 on paraquat plates did not differ significantly between MA and AC lines ($p = 0.363$; data not shown).

When summed over the entire experiment duration, the proportion of nematodes that ever reached adulthood on NGM plates was virtually identical between MA and AC lines (MA vs. AC, $p = 0.974$, presented by line on Figure 6). In contrast, the proportion of nematodes that ever reached adulthood on paraquat stress was slightly, but not significantly higher in MA lines than in AC lines (MA vs. AC, $p = 0.145$, Figure 6). To assess the delayed development resulting from paraquat, we used the development score (maximum score = 50, indicating 10 live, adult nematodes). We assessed development score at day 3 because adults were removed from NGM plates after day 3. There were no significant differences between the development scores of MA and AC nematodes on NGM plates ($p = 0.796$; data presented by line, Figure 7) or on paraquat plates ($p = 0.1969$; Figure 7). The comparison of development score on NGM versus paraquat plates allows us to comment on relative susceptibility to stress. The decrement in development score was greatest for MA line 523 (54% decrease on paraquat versus NGM) and smallest for MA line 553 (14%). The decrement in development score for N2 AC was intermediate (46%).

As summarized in Section A, HK104 nematodes exposed to paraquat had significantly lower survival than did nematodes on NGM plates. HK104 AC and MA nematode survival did not change over time on NGM plates (Table 3). The average proportion of nematodes surviving on paraquat plates did not decrease over time in MA or AC lines (as stated above, assess temporal changes from day 2; Figure 8). Since most nematodes on NGM plates were adults on day 4, we assessed survival at day 4 (Table 3). On NGM plates, MA lines tended to have higher survival to day 4, but the difference in survival between MA and AC lines was not significant (MA vs. AC, $p = 0.777$, Table 3).

On paraquat plates, AC nematodes had higher survival than did nematodes from any MA line, but the difference between MA and AC was not significant (MA vs. AC, $p = 0.162$, Figure 8 and Table 3). Across all time points, MA line 261 had the lowest survival on paraquat plates (Table 3).

As summarized in Section A, HK104 nematodes exposed to paraquat had significantly slower development than did nematodes on NGM plates. The number of days for the first adult to appear on the NGM plates was similar between MA and AC lines (AC: 3.6 ± 0.25 days, MA: 3.8 ± 0.2 days, MA vs. AC, $p = 0.4844$, data not shown). The proportion of hatched nematodes that reached adulthood on day 4 on NGM plates ranged from 0.59-0.8 (MA vs. AC, $p = 0.611$; data not shown). Nematodes from one MA line (232), never reached adulthood on paraquat plates. Nematodes from the other two MA lines and the HK104 AC lines needed, on average, two days longer to reach adulthood on paraquat than on NGM plates (MA: 6.62 ± 0.06 days, HK104 AC: 5.75 ± 0.48 days, MA vs. AC, $p = 0.2433$; data not shown). On day 6, the first day any adult nematodes were found on paraquat plates, the proportion of nematodes within an MA or AC line that were adults ranged from 0 – 0.3 (data not shown). The proportion of nematodes that were adults on day 6 on paraquat plates did not differ significantly between MA and AC lines (MA vs. AC, $p = 0.229$; data not shown). When summed over the entire experiment duration, the proportion of HK104 nematodes that ever reached adulthood on NGM plates was very similar between MA and AC lines (MA vs. AC, $p = 0.839$, presented by line on Figure 9). In contrast, the proportion of nematodes that ever reached adulthood on paraquat stress was higher in AC lines than in MA lines (MA vs. AC, $p = 0.084$, Figure 9). Development scores did not differ significantly between MA

and AC lines on day 3 on NGM (MA vs. AC, $p = 0.368$; Figure 10) or paraquat plates (MA vs. AC, $p = 0.562$; Figure 10). The decrement in development score was greatest for MA line 261 (42% decrease on paraquat versus NGM) and smallest for MA line 232 (15%). The decrement in development score for HK104 AC was intermediate (30%).

Unlike the heat assay, in which HK104 lines survived the stress treatment better than the N2 AC lines, in the paraquat assay, HK104 and N2 had similar survival, but HK104 MA and AC lines had much slower development than N2 AC lines. For example, nematodes from the N2 AC lines needed an average of 4.9 days to reach adulthood on paraquat, while the fewest average days needed for any HK104 line was 5.75 (HK104 AC). Additionally, on day 5, 50% of the N2 AC nematodes had reached adulthood on paraquat plates, but <1% of any HK104 nematodes were adults. Finally, one of the HK104 MA lines (232) had high survival but no individuals reached adulthood on any paraquat plate.

D. Salt Assay – Experiment 1

The salt stress assays for experiment 1 were conducted in two blocks, the N2 sequenced lines were assayed in block 1 and the HK104 sequenced lines in block 2. Eggs from each AC and MA line were placed on wells of NGM agar with a high concentration of salt or a well of normal NGM agar, and development and survival were monitored for up to eight days. We did not detect any among-line variation in N2 or HK104 AC for any trait related to salt stress. While we did detect among-line variation for some traits in the N2 and HK104 MA lines, the variance was never significantly different from zero and therefore is not presented here. Patterns in survival only reflect survival of hatched nematodes, as described above.

As summarized in Section A, N2 nematodes exposed to salt had significantly lower survival than did nematodes on NGM. AC and MA nematode survival did not change over time on NGM plates (Table 4). The average proportion of nematodes surviving on salt did not decrease over time in MA or AC lines (Figure 11). Since most nematodes on NGM were adults on day 3, we assessed survival at day 3 (Table 4). While AC nematodes tended to have higher survival than MA nematodes on both NGM and salt, these MA treatment differences were not significant (NGM: MA vs. AC, $p = 0.289$, Table 4; paraquat: MA vs. AC, $p = 0.376$, Figure 11 and Table 4). Across all time points, MA line 526 had the highest survival on paraquat and MA line 523 had the lowest survival (Table 4).

As summarized in Section A, N2 nematodes exposed to salt had significantly slower development than did nematodes on NGM. Nearly all nematodes reached adulthood on day 3 on NGM; across MA and AC lines, the proportion of nematodes that were adults on day 3 ranged from 0.5 – 0.9 (data not shown). Nematodes on salt, on average, needed an additional 2.5 days to reach adulthood. The number of days to the first adult appearing on salt was higher for MA nematodes than AC nematodes (MA, 5.3 ± 0.1 days, AC: 5.1 ± 0.2 days, MA vs. AC, $p = 0.329$; data not shown). On day 5, the first day any adult nematodes were found on salt, the proportion of nematodes within an MA or AC line that were adults ranged from 0.33 – 0.68. The proportion of nematodes that were adults on day 5 on salt did not differ significantly between MA and AC lines ($p = 0.252$; data not shown). When summed over the entire experiment duration, the proportion of AC nematodes that ever reached adulthood on NGM was slightly higher than that of MA nematodes (MA vs. AC, $p = 0.172$, presented by line on Figure 12).

Significantly more AC nematodes than MA nematodes reached adulthood on salt (MA vs. AC, $p = 0.0497$, Figure 12).

To assess the delayed development resulting from salt, we used the development score (maximum score on NGM = 50, indicating 10 live, adult nematodes; maximum score on salt = 25, indicating 5 live, adult nematodes). We assessed development score at day 3 because adults were removed from NGM wells after day 3. There were no significant differences between the development scores of MA and AC nematodes on NGM ($p = 0.117$; data presented by line, Figure 13) or on salt ($p = 0.177$; Figure 13). The decrement in development score was greatest for MA line 523 (89% decrease on salt versus NGM) and smallest for MA line 526 (61%). The decrement in development score for N2 AC was intermediate (78%).

As summarized in Section A, HK104 nematodes exposed to salt had significantly lower survival than did nematodes on NGM. HK104 AC and MA nematode survival did not change over time on NGM (Table 4). The average proportion of nematodes surviving on salt did not change substantially over time in MA or AC lines (as stated above, assess temporal changes from day 2; Figure 14 and Table 4). Since most nematodes on NGM were adults on day 3, we assessed survival at day 3 (Table 4). On NGM, AC nematodes tended to have higher survival to day 3, but the difference in survival between MA and AC lines was not significant (MA vs. AC, $p = 0.289$, data not shown). On salt, MA nematodes had higher survival than did AC nematodes, but the difference between MA and AC was not significant (MA vs. AC, $p = 0.128$, Figure 14). Across all time points, MA line 261 had the lowest survival on salt (Table 4).

As summarized in Section A, HK104 nematodes exposed to salt had significantly slower development than did nematodes on NGM. The number of days for the first adult to appear on NGM was identical between MA and AC lines (both MA and AC: 3.0 ± 0 days, data not shown). The proportion of hatched nematodes that reached adulthood on day 3 on NGM ranged from 0.53-0.9 (MA vs. AC, p = 0.999; data not shown). MA nematodes and the HK104 AC lines needed, on average, nearly four days longer to reach adulthood on salt than on NGM (MA: 6.74 ± 0.3 days, HK104 AC: 6.0 ± 1.1 days, MA vs. AC, p = 0.518; data not shown). On day 5, the first day any adult nematodes were found on salt, the proportion of nematodes within an MA or AC line that were adults ranged from 0 – 0.1 (data not shown). The proportion of nematodes that were adults on day 5 on salt did not differ between MA and AC lines (data not shown). When summed over the entire experiment duration, a greater proportion of AC nematodes than MA nematodes ever reached adulthood on NGM (MA vs. AC, p = 0.318, presented by line on Figure 15). In contrast, the proportion of nematodes that ever reached adulthood on salt was higher in MA lines than in AC lines (MA vs. AC, p = 0.066, Figure 15). Development scores of AC and MA nematodes did not differ significantly on day 3 on NGM (MA vs. AC, p = 0.217; Figure 16) or on salt (MA vs. AC, p = 0.138; Figure 16). The decrement in development score was greatest for MA line 261 (95% decrease on salt versus NGM) and smallest for MA line 232 (50%). The decrement in development score for HK104 AC was very high (98%).

In the salt assay, the survival and development of HK104 was substantially lower than that of the N2 AC run concurrently. For example, on day 3, 50% of the N2 AC nematodes were alive on salt while the highest survival among HK104 lines was 35%

(MA line 263). Additionally, N2 AC nematodes required an average of 5.5 to develop to adulthood on salt while the HK104 nematodes required 6-7 days. Finally, overall survival to adulthood for N2 AC nematodes on salt was 0.4 ± 0.1; the highest surviving HK104 line averaged 0.12 ± 0.04 (MA line 263).

E. Correlation with Base Substitution Rate

In experiment 1 we tested whether physiological robustness to stress of the sequence N2 or HK104 lines would correlate with base substitution rate. None of the measurements taken on nematodes exposed to heat, paraquat, or salt stresses significantly correlated with either the 2009 N2 base substitution rate estimates nor the 2012 N2 and HK104 base substitution rate estimates.

F. Effectiveness of Stress Assays and Block Effect -Experiment 2

Across all three assay types, nematodes from the second-order MA experiment tended to have lower survival and/or slower development in stress conditions than did nematodes in the respective control treatments. Data summaries presented in this section are averaged across MA line and across MA treatment and most are not presented in figures or tables. These data will be described in greater detail in subsequent sections. For reasons that are unclear (see Discussion), nematode survival and/or development in all three stress assays differed across blocks. To illustrate the differences between blocks, line means and standard errors for the N2 AC nematodes (the only nematodes assayed in both blocks) of several traits are presented in Table 5. Differences between blocks are detailed below. In all cases, MA versus AC comparisons were conducted with the respective block N2 AC data, not with AC data summed across block.

In both blocks of the heat assay, nematode survival in both control conditions (20°C) and heat stress conditions (35°C) was measured at 18 – 20 hours. Across block, all nematodes exposed to heat had significantly lower survival than did nematodes in control conditions (survival in control, 0.91 ± 0.02, survival in heat, 0.40 ± 0.09, control vs. heat, $p = 0.000082$; data not shown). The N2 AC lines performed poorly in block 2 in comparison to block 1 in the heat stress treatment but performed similarly in the control conditions (Table 5).

Nematodes exposed to paraquat had significantly lower survival to adulthood than did nematodes on NGM plates (control conditions). In both blocks of the paraquat assay, survival to adulthood was significantly lower in nematodes exposed to paraquat (proportion survived to adulthood, 0.217 ± 0.05) than in nematodes on NGM plates (0.497 ± 0.06; paraquat vs. NGM, $p = 0.0006$; data not shown). Additional traits will be presented below in Section H. The N2 AC lines performed poorly in block 2 in comparison to block 1 on both NGM and paraquat-containing plates (Table 5).

Nematodes exposed to salt had significantly lower survival to adulthood than did nematodes on NGM plates. In both blocks of the salt assay, survival to adulthood was significantly lower in nematodes exposed to salt (proportion survived to adulthood, 0.14 ± 0.03) than in nematodes on NGM plates (proportion survived to adulthood, 0.405 ± 0.08; salt vs. NGM, $p = 0.0039$; data not shown). Additional traits will be presented below in Section I. The N2 AC lines performed poorly in block 2 in comparison to block 1 on both NGM and high salt agar (Table 5).

G. Heat Assay – Experiment 2

The heat stress assays for experiment 2 were conducted in two blocks, with the N2 ancestral control assayed in each block, and lines from the low and high fitness second order experiment randomly assigned to block, with low and high fitness categories represented in each block. In both assays, young adults were placed on NGM agar plates pre-heated to 35°C and 20°C, and survival was monitored for up to 25 hours.

In both blocks, the 18 hour point was the only time point at which both the 35°C heat stress and 20°C control plates were checked. In the 18 hours in control conditions, the proportion of nematodes alive differed significantly by fitness category (fitness, $p = 0.0494$), but did not differ by block (block, $p = 0.130$; interaction, $p = 0.626$). Since the block effect was not significant, the data were analyzed by one-way ANOVA, in which fitness category was not significant (fitness, $p = 0.11$). In the 18 hour control conditions, the proportion of AC nematodes and high fitness MA nematodes were very similar and slightly higher than the proportion of low fitness MA nematodes that were alive (18H Control on Figure 17, Blocks 1 and 2 on 18). Mutational declines in ability to survive 18 hours of control conditions ($\Delta M \times 10^3$) were greater in low fitness MA lines ($\Delta M = -0.204$) than in high fitness lines ($\Delta M = 0.096$; Table 6). The positive ΔM in the high fitness lines indicates that most of the high fitness MA line means were larger than the AC line mean, which suggests that there was no mutational decline in this trait in the high fitness MA lines. The negative ΔM value for the low fitness MA lines indicates a mutational decline in this trait. Since one ΔM value was positive and the other negative, we can consider these ΔM values to be significantly different from each other (i.e., mutational decline in survival was significantly greater in low fitness MA lines than in high fitness MA lines).

After 18 hours of heat stress, the proportion of nematodes alive differed significantly by fitness category (fitness, $p = 0.011$) but not by block (block, $p = 0.125$; interaction, $p = 0.002$). The strong effect of the interaction was driven solely by the high survival of the AC nematodes in block 1 (Table 6). Since the block term was not significant, the data were analyzed by one-way ANOVA; AC nematodes had significantly higher survival after 18 hours of heat stress than did high fitness MA nematodes (high fitness, $p = 0.049$) and low fitness MA nematodes (low fitness, $p = 0.026$; 18 H Heat on Figure 17, both blocks on Figure 19). The ΔM ($x\ 10^3$) values for survival of 18 hours of heat stress were indistinguishable between fitness categories, (high fitness, $\Delta M = -1.484$; low fitness, $\Delta M = -0.761$; Table 6).

The 23 hour and 25 hour time points were assayed in block 1 only. In both time points, AC nematodes had significantly higher survival than did nematodes from high or low fitness MA lines (all comparisons, $p \leq 0.000524$; Figure 17 and Table 6). The ΔM values for survival to 23 and 25 hours ranged from $\Delta M = -3.052$ to -3.829 and were slightly greater in the low fitness MA lines than in the high fitness MA lines (Table 6).

H. Paraquat Assay – Experiment 2

The paraquat stress assays for experiment 2 were conducted in two blocks, with ancestral control and randomly selected high and low fitness MA lines in each block. Eggs from each AC and MA line were placed on agar plates containing paraquat or without paraquat (NGM, control conditions); survival and development were monitored for up to eight days. Patterns in survival only reflect survival of hatched nematodes. Hatching success was not analyzed because we cannot determine whether eggs did not

hatch due to stress treatment or because they were infertile eggs (some MA lines are more prone to laying infertile eggs; Baer, pers. comm.).

As summarized in Section F, nematodes exposed to paraquat had significantly lower survival than did nematodes on NGM plates. AC and MA nematode survival did not change over time on NGM plates (Table 7). The average proportion of AC and MA nematodes surviving on paraquat plates did not decrease over time when analyzed across blocks (Figure 21). Since most nematodes on NGM plates were adults on day 3, we assessed survival at day 3 (Table 7). On NGM plates, both fitness category and block affected survival to day 3 (fitness, $p = 0.00014$; block, $p < 0.00001$; interaction, $p = 0.109$). In block 1, AC and high fitness MA survival were indistinguishable and greater than low fitness nematode survival ($p > 0.05$; block 1 on Figure 20). The ΔM ($x\ 10^3$) for survival to day 3 on NGM was $\Delta M = -0.222$ for high fitness lines and $\Delta M = -1.33$ for low fitness lines (Table 7). In block 2, overall survival was lower and the relative rankings between fitness categories were altered. In block 2, high fitness MA nematodes had the highest survival and the AC and low fitness nematodes were indistinguishable ($p > 0.05$; block 2 on Figure 20). The ΔM values in block 2 were $\Delta M = 0.93$ for high fitness MA lines and $\Delta M = -1.39$ for low fitness MA lines. When averaged across blocks, but calculated with the respective block controls, the ΔM for survival to day 3 on NGM was $\Delta M = 0.239$ for high fitness MA lines and $\Delta M = -1.37$ for low fitness MA lines; these ΔM values indicate that low fitness MA nematodes had significantly greater mutational declines in survival to day 3 on NGM than did high fitness MA nematodes.

On paraquat plates, block (and the interaction term) affected survival to day 3, but survival did not differ significantly between fitness categories (fitness, $p = 0.224$; block, p

< 0.0001; interaction, 0.0004; Table 7 and Day 3 on Figure 21). In block 1, AC nematodes had the higher survival than either set of MA nematodes; survival of high and low fitness MA nematodes were indistinguishable (Block 1 on Figure 22). The ΔM for survival to day 3 on paraquat was ΔM = -0.699 for high fitness MA lines and ΔM = -1.29 for low fitness lines. In block 2, low fitness MA nematodes had the highest survival (Block 2 on Figure 22). Since the proportion of AC nematodes that survived to day 3 was abnormally low (75% lower in block 2 than in block 2; Table 7) and lower than all but one (high fitness) MA line (Table 7), the ΔM values for both fitness categories are positive (high fitness, ΔM = 0.316; low fitness, ΔM = 2.88). When averaged across block, ΔM for survival to day 3 on paraquat was ΔM = -0.293 for high fitness lines and ΔM = 1.21 for low fitness lines.

As summarized in Section F, nematodes exposed to paraquat had significantly slower development than did nematodes on NGM. Nearly all nematodes reached adulthood on day 3 (block 1) or day 4 (block 2) on NGM (Figure 23), the number of days needed to develop to adulthood did not vary between fitness categories (fitness, $p = 0.53$) but did differ between blocks (block, $p < 0.0001$; interaction, $p = 0.53$). Across fitness categories, the proportion of nematodes that were adults on day 3 or 4 (Figure 24) ranged from 0.46 – 0.92 (block 1) and from 0.28 – 1 (block 2). In block 1, the proportion of nematodes that reached adulthood did not vary across fitness category (fitness, $p = 0.66$; block 1 on Figure 24). In block 2, the proportion of nematodes that reached adulthood on NGM varied significantly across fitness category (fitness, $p = 0.012$), significantly more high fitness MA nematodes reached adulthood on day 4 than did low fitness MA nematodes (high vs. low, $p < 0.05$; block 2 on Figure 24).

Nematodes on paraquat, on average, needed an additional 2 days to reach adulthood (Figure 25); the necessary number of days varied significantly by block (block, $p = 0.0002$) but not by fitness category (fitness, $p = 0.43$; interaction, $p = 0.38$). In block 1, low fitness MA lines needed slightly more time to develop to adulthood than did AC and high fitness MA nematodes (block 1 on Figure 25). In block 1, the ΔM for high fitness MA lines was $\Delta M = 0.013$, the ΔM value for low fitness MA lines was $\Delta M = 0.429$ (Table 8). For this trait, a negative ΔM value indicates that nematodes from a MA fitness category needed fewer days to develop to adulthood. Nematodes assayed in block 2 tended to require more days to develop to adulthood than did nematodes in block 1. In block 2, AC nematodes needed more days to develop than did nematodes from either MA fitness category. The ΔM for high fitness MA lines was $\Delta M = -0.336$ and the ΔM for low fitness MA lines was $\Delta M = -0.465$ in block 2. When averaged across blocks, the lack of significant fitness category becomes apparent; for high fitness MA lines, $\Delta M = -0.127$ and for low fitness MA lines, $\Delta M = -0.108$. On day 5, the first day any adult nematodes were found on paraquat, the proportion of nematodes within a fitness category that were adults (Figure 26) ranged from 0.22 – 0.88 (block 1) and from 0.04 – 0.16 (block 2). The proportion of nematodes that were adults on day 5 on paraquat plates varied significantly by fitness category (fitness, $p = 0.03$) and block for the AC and high fitness categories (block, $p < 0.001$; interaction, $p = 0.01$). In block 1, significantly more AC nematodes were adults on day 5 than were low fitness nematodes (AC vs. low, $p = 0.001$; block 1 on Figure 26). The proportions of nematodes that were adults did not vary among fitness categories in block 2 (all comparisons, $p \geq 0.79$; block 2 on Figure 26). The ΔM for proportion of nematodes that were adults on day 5 are presented in Table 8; block 2 ΔM

and combined blocks 1 and 2 ΔM values reflect the very low proportion of AC adults on day 5 (proportion of AC nematodes that were adults, 0.04 ± 0.14).

When summed over the entire experiment duration, the proportion of nematodes that ever reached adulthood on NGM (Figure 27) varied by fitness category (fitness, $p = 0.0007$) and by block (block, $p = 0.008$; interaction, $p = 0.56$; Figure 28). There was no consistent change in proportion surviving to adulthood across block on NGM (Figure 28). In general, the proportion of nematodes that survived to adulthood was lowest in the low fitness MA lines; this difference was significant in block 2 (high vs. low, $p = 0.013$; Figures 27 and 28). When calculated within and across blocks, ΔM values for survival to adulthood on NGM were positive in high fitness MA lines and negative (i.e., significantly lower) in low fitness MA lines (Table 8). On paraquat plates, the proportion of nematodes that ever reached adulthood varied by fitness category (fitness, $p < 0.0001$; Figure 27) and by block (block, $p < 0.0001$; interaction, $p = 0.0009$; Figure 29). The block effect was entirely driven by the significant decrease in survival of the AC nematodes in block 2 versus block 1 (AC in block 1 vs. block 2, $p = 0.0001$). In block 1, the low fitness MA lines had significantly lower survival to adulthood than did the AC nematodes (AC vs. low, $p = 0.0001$) and the high fitness MA nematodes (high vs. low, $p = 0.0003$; block 1 on Figure 29). In contrast, all nematodes in block 2 had similar survivorship to adulthood (block 2 on Figure 29). Mutational declines in survivorship on paraquat were greater in low fitness MA lines than in high fitness MA lines when calculated within and across blocks (Table 8).

To assess the delayed development resulting from exposure to paraquat, we used the development score (maximum score = 50, indicating 10 live, adult nematodes). We

assessed development score at day 3 because adults were removed from NGM plates after day 3 (Figure 30). Development score on day 3 on NGM differed only by block (block, $p = 0.04$; fitness, $p = 0.15$; interaction, $p = 0.11$); the block effect was driven by the increase in development score in high fitness MA lines in block 2 (Figure 30). Development scores did not differ significantly by fitness category in block 1. In block 2, high fitness MA nematodes had significantly higher development scores than did low fitness MA nematodes (high vs. low, $p = 0.017$). Within and across blocks, the ΔM values for development score on NGM tended to be more negative in low fitness lines than in high fitness lines (Table 8). Development scores of nematodes on paraquat plates tended to decrease over time (Figure 31); and varied significantly by block (block, $p < 0.001$; interaction, $p < 0.001$) but not by fitness category (fitness, $p = 0.21$). Unlike the block pattern on NGM plates, development scores overall were lower in block 2 than in block 1 (Figure 32). In block 1, development scores varied significantly across the three fitness categories (all comparisons, $p \leq 0.045$) and followed the predicted pattern based on fitness category (Figure 32). Development scores did not differ significantly across fitness categories in block 2 (all comparisons, $p \geq 0.09$). Given the poor performance of the AC nematodes in block 2, ΔM values for development score on paraquat have no consistent pattern (Table 8). Decrements in development score are uninformative because development scores tended to be higher on paraquat plates than on NGM plates (data not shown; can be illustrated by comparing Figures 30 and 32).

I. Salt Assay – Experiment 2

The salt stress assays for experiment 2 were conducted in two blocks, with ancestral control and randomly selected high and low fitness MA lines in each block.

Eggs from each AC and MA line were placed on wells containing high salt agar or NGM (control conditions); survival and development were monitored for up to eight days. Patterns in survival only reflect survival of hatched nematodes.

As summarized in Section F, nematodes exposed to salt had significantly lower survival than did nematodes on NGM. Nematode survival did not change over time on NGM (Table 9). The average proportions of nematodes surviving on salt did not decrease over time in any consistent manner within or across fitness category (Table 9 and Figure 33). Since most nematodes on NGM were adults on day 3, we assessed survival at day 3 (Table 9). On NGM, survival to day 3 varied by block (block, $p < 0.001$) but not by fitness category (fitness, $p = 0.78$; interaction, $p = 0.11$). Survival on NGM was significantly lower in block 2 than in block 1 in all fitness categories (all comparisons, $p \leq 0.01$; Figure 34). The ΔM values ($\times 10^3$) for survival to day 3 on NGM indicate greater mutational decline in survival on NGM in block 1 and the combined blocks, but not in block 2 alone (Table 9).

On salt, survival to day 3 varied significantly by fitness category (fitness, $p < 0.001$; interaction, $p = 0.002$) but not by block (block, $p = 0.29$). Survival on salt stress was graphed for days 2 through 5 on Figure 33. Significant differences between fitness categories were detected only in block 1; nematodes from high fitness MA lines had significantly higher survival to day 3 on salt than did nematodes in other fitness categories (all comparisons, $p < 0.001$; Figure 35). Within and across blocks, mutational declines in survival to day 3 on salt were smaller or more negative in low fitness lines than in high fitness lines; this trend continued on days 4 and 5 (Table 9).

As summarized in Section F, nematodes exposed to salt had significantly slower development than did nematodes on NGM. Nearly all nematodes reached adulthood on day 3 on NGM (data not shown); the number of days needed to develop to adulthood did not vary between fitness categories or blocks (all $p > 0.05$). Across fitness categories, the proportion of nematodes that were adults on day 3 (Figure 36) ranged from 0.0.26 – 1.0 (block 1) and from 0.4 – 1.0 (block 2); the proportion of nematodes that were adults did not differ between fitness categories ($p = 0.703$).

Nematodes on salt, on average, needed an additional 2 - 4 days to reach adulthood (Figure 37); the necessary number of days varied significantly by fitness category (fitness, $p = 0.008$; interaction, $p = 0.034$) but not by block (block, $p = 0.18$). The only significant difference in the time needed to develop to adulthood was detected in block 1, in which low fitness nematodes needed significantly more days to develop than did high fitness nematodes (low vs. high, $p < 0.001$; Figure 37). For this trait, a positive ΔM value indicates that the MA nematodes developed more slowly (needed more days) than did the AC nematodes. Within and across block, the ΔM values indicate that low fitness nematodes experienced a greater mutational change in the amount of time needed to develop (Table 10). On day 5, the first day any adult nematodes were found on salt, the proportion of nematodes within a fitness category that were adults (Figure 38) ranged from 0.0 – 0.11 (block 1) and from 0.0 – 0.28 (block 2). The proportion of nematodes that were adults on day 5 on salt plates varied significantly by fitness category (fitness, $p < 0.001$) and block (block, $p < 0.001$; interaction, $p = 0.08$). The proportions of nematodes that were adults did not vary among fitness categories in block 1 (block 1 on Figure 38). In block 2, significantly fewer low fitness nematodes were adults on day 5 than were high

fitness and AC nematodes (all comparisons, $p < 0.001$; block 2 on Figure 38). The ΔM values within and across blocks indicate that the mutational declines in the ability to develop to adulthood by day 5 on salt were greater in the low fitness lines than in the high fitness lines (Table 10).

When summed over the entire experiment duration, the proportion of nematodes that ever reached adulthood on NGM (Figures 39) varied by block (block, $p < 0.001$) but not by fitness (fitness, $p = 0.34$; interaction, $p = 0.20$; Figure 40). The total proportion surviving to adulthood on NGM tended to be lower in block 2 than in block 1 (significant only for high fitness MA lines, block 1 vs. block 2, $p < 0.001$). In general, nematodes from low fitness lines had lower survival to adulthood than did nematodes in other fitness categories, as indicated in the ΔM values (Table 10). On salt, the proportion of nematodes that ever reached adulthood varied by fitness category (fitness, $p < 0.001$; interaction, $p = 0.01$ Figure 40) but not by block (block, $p = 0.08$; Figure 41). In block 1, the high fitness MA lines had significantly higher survival to adulthood than did the AC and low fitness nematodes (both comparisons, $p \leq 0.009$). In block 2, the low fitness nematodes had the lowest proportion surviving to adulthood (both comparisons, $p < 0.001$). Mutational declines in survivorship on salt were greater in the low fitness MA than in high fitness MA lines (Table 10).

To assess the delayed development resulting from exposure to salt, we used the development score (maximum score on NGM = 50, indicating 10 live, adult nematodes). We assessed development score at day 3 because adults were removed from NGM wells after day 3 (Figure 42). Development score on day 3 on NGM differed by block (block, $p < 0.001$; fitness, $p = 0.48$; interaction, $p = 0.10$) but did not differ consistently by fitness

category (Figure 42), as indicated by the ΔM values (Table 10). Development scores on salt (maximum score on salt = 25, indicating 5 live, adult nematodes) differed significantly by fitness category (fitness, $p < 0.001$; Figure 43) and block (block, $p = 0.031$; interaction, $p = 0.002$; Figure 44). In block 1, development scores of the high fitness lines were significantly greater than those of AC and low fitness nematodes (both comparisons, $p < 0.001$; Figure 44). In block 2, the development scores were indistinguishable across fitness category ($p \geq 0.12$). The ΔM values indicate that mutational changes in development score on salt tended to be greater in low fitness lines than in high fitness lines (Table 10). Development scores of nematodes exposed to salt were significantly lower than those of nematodes on NGM ($p < 0.05$).

J. Changes in Motility and Body Size – Experiment 2

Changes in motility and body size were assessed for 15 nematodes from each second order MA line; the N2 AC line was divided into five pseudolines, 15 nematodes from each AC pseudoline were assayed. Assays were conducted in two blocks (independent from the other stress assays), with high and low fitness and five AC pseudolines represented in each block. In each set of 15 nematodes, five nematodes were placed individually on NGM plates, five nematodes were placed individually on paraquat-containing plates, and five nematodes were placed individually on high salt plates. Nematodes were monitored twice a day for four days. The data presented here reflect changes in motility between days 1 and 5 and the body size measurements at 48 hours, the time point at which the most nematodes were found.

Body sizes were more heavily influenced by block than were motility measures. The data analyzed were proportional changes in body size from the time 0 measurement

to the 48 hour measurement (data not shown). Nematodes in all fitness categories and on all plate types tended to increase in size over the 48 hours (data not shown). In block 1, body size changes were larger on paraquat plates than on NGM plates; increases in body size were more variable on salt plates. In general, nematodes from high and low fitness grew more than the AC nematodes on the respective plate types, as indicated by the ΔM values (Table 11). In block 2, the trends were qualitatively different, in that nematodes increased in size over time, but MA nematodes grew less than AC nematodes grew, as indicated by the ΔM values. Averaged across blocks, it is unclear whether there is a consistent relationship between body size changes and fitness category.

Motility, estimated as the total surface area contacted by a nematode in a 10-second period (Table 12), differed significantly across plate types (NGM, paraquat, salt; plate, $p < 0.0001$; interaction, $p = 0.015$) but not by time (time 0 versus 48 hours, $p = 0.286$). Nematode motility was higher on paraquat plates than on any other plate type (all comparisons, $p \leq 0.043$) and decreased over time. Nematode motility was indistinguishable on salt and NGM plates; nematode motility tended to increase over time on salt plates but not on NGM plates. Nematodes from the three fitness categories had similar motility on NGM plates at time 0 but by 48 hours the motility of the MA nematodes was lower than that of the AC nematodes, as indicated by the ΔM values (Table 12). On the other two plate types, nematodes from low fitness lines tended to have the lowest motility, as indicated by the greatest mutational declines in motility across plate type and day (Table 12).

DISCUSSION

In this study, we tested whether physiological robustness (i.e., response to a stressor) differed by nematode strain (experiment 1) and whether it correlated with base substitution rate (experiment 1) or fitness category (inversely related to estimated mutation rate; experiment 2). In experiment 1, we found that physiological responses do not correlate with base substitution rates. We can think of several reasons why we did not detect significant correlations between stress traits and base substitution rates. First, it is possible that the types of mutations characterized in the sequencing studies (Denver *et al.* 2009 versus Denver *et al.* 2012, in review) have little to no effect on these types of traits. Since, to our knowledge, this type of study (comparing base substitution rates to physiological traits) has not been conducted in nematodes, we cannot assess this possibility in the context of published literature. The closest comparison would be the characterization of chemotactic behavior in MA lines from a different N2 MA experiment (Ajie *et al.* 2005; Estes *et al.* 2005). In those studies, which were published as accompanying manuscripts, nematode movement away from a repellent was tracked over time; nematodes from MA lines had lower velocity of movement and slower turns than did ancestor (AC) nematodes. However, base substitution rates are not available for the MA lines they assayed.

Second, it is possible that our sample size (5 MA lines in N2, 3 MA lines in HK104) was too small to detect significant correlations of line means (i.e., too few line means) and/or that the amount of variance within MA lines was larger than the differences between line means and larger than the difference between MA and AC. One of the limitations of working with mutation accumulation lines is that the nematodes in

each MA line, particularly those lines that have low fitness, are difficult to maintain in the lab. We assayed all but one of the sequenced N2 lines but only were able to grow three of the seven sequenced HK104 lines. Since sequencing technology is rapidly improving and sequencing projects keep growing larger (note that in three years time, the number of lines sequenced in a single study more than doubled and multiple genotypes per MA line were sequenced; Denver *et al.*, 2009 versus 2012), this question could be re-addressed in several years, with a larger number of MA lines and a focus on those traits that were closest to having a significant relationship with base substitution rate. In terms of the potentially confounding influence of within-line variance, it is well-established that mutational effects are larger when measured under stress (Kondrashov and Houle 1994; Fry *et al.* 1996) and that mutational variance (variance within MA lines divided by the AC mean) increases when traits are measured in stressful conditions (Vassilieva *et al.* 2000). We detected a similar trend across traits, the within-line variance tended to be larger in nematodes exposed to stress, which constrains our ability to detect significant MA versus AC trends and correlations with base substitution rates.

Third, there is a surprising lack of congruence between estimations of base substitution rates in the N2 sequenced lines (Denver *et al.* 2009 versus Denver *et al.* 2012, in review) that complicates our attempt to correlate physiological robustness with base substitution rate. At the time this project was initiated, the first N2 paper had been published and the results published in the 2012 paper were in progress. The two papers present very different views of the mutational properties of the N2 MA lines. The base substitution rates presented in both papers for a given N2 MA line do not at all correlate ($r = -0.361$, $p = 0.484$; calculation conducted using published base substitution rates) and

are significantly lower in the second (2012) publication than in the first (2009). In the 2012 paper, Denver and colleagues sequenced multiple isolates from each of 5 - 7 MA lines (generating error estimates) from each of four strains in *C. elegans* and *C. briggsae*. They found that base substitution rates do not differ within strain or even across strain. These results indicate that there are still a number of limitations in mutation detection methods, which is not unexpected, given that the technology is less than ten years old. Additionally, mutational declines in fitness of the N2 and HK104 lines did not correlate with the base substitution rates (2009 and 2012 base substitution rates versus mutational declines in fitness as reported in 2005, $p > 0.500$; Baer *et al.* 2005; Denver *et al.* 2009; Denver *et al.* 2012, in review). Denver and coworkers only measured the base substitution rate and not the other categories of mutation (e.g., large deletions); it is possible that one cannot explain the relationship between mutational declines in fitness and genome quality without measuring more mutation types. Given this lack of congruence between sequencing studies and the lack of fit between base substitution rate and mutational decline in fitness, it may have been premature to attempt to correlate physiological robustness and mutation rate, given only the estimates of base substitution rate.

What did surprise (and please) us, though, is that we were able to detect differences between the *C. elegans* (N2) and *C. briggsae* (HK104) strains, particularly given that we assayed such a small number of HK104 MA lines. The Denver sequenced paper (2012) presented the first recorded base substitution rates for the HK104 MA lines, which were indistinguishable from those of the N2 MA lines. This homogeneity across strain was in contrast to evidence found in Baer (2005), which demonstrated clear

differences in productivity of MA lines from the two strains (N2 > HK104). Our work aligns better with the Baer *et al.* 2005 fitness data than with the sequencing data and adds further evidence to our concern that base substitution rates (alone) cannot be used to predict physiological robustness (or vice versa).

In contrast to the N2 and HK104 sequenced lines, we were able to predict fitness category in the second order MA lines by measuring physiological robustness to environmental stress. Within a stress assay, it was considerably easier to distinguish any high fitness MA line from any low fitness MA line than it was to compare a high or low fitness MA line to another MA line in the same fitness category. When experiments 1 and 2 are compared, it becomes clear that using stress assays to predict fitness is more successful when there are large differences in fitness between the lines than when there are subtle differences in fitness. Fitness of the sequenced N2 lines (experiment 1) varied by 1.5 – 29% from the MA mean fitness; in contrast, fitness of the N2 second-order MA lines (experiment 2) varied by 22 – 72% from the MA mean fitness. The relationship between stress and fitness is a promising area of study and our work here suggests that we can predict relative differences in mutational declines in fitness by characterizing susceptibility to stress.

The relationship between stress response, fitness category, and future mutation rates (the second component of my experiment 2 hypotheses) were not presented in the Results section because our collaborator, Baer, has had to redo the fitness assays of select second order MA lines. As such, we can only comment qualitatively on this potential relationship, and do so in second order results portion of the Discussion. Since we do not have the final results from Baer's assay, in our discussion we use ΔM values for stress

traits and the ΔM values for most of the second-order lines (provided to us by Baer) to demonstrate the differences in high and low fitness MA lines. These values allow us to evaluate the degree to which stress response is related to mutational declines in fitness (MA generations 250 – 375 in Baer's assay) without formally calculating the covariance between the traits (stress and fitness).

Efficacy of the Stress Treatments

As described in the Results sections A and F, the stress assays were effective at stressing nematodes from both strains and all sets of MA lines in experiments 1 and 2. Across traits, nematodes in stress treatments tended to have decreased survival and/or slower development. We optimized our stressor treatments methods on previous studies, particularly those that utilized N2 nematodes, but in our preliminary experiments we tended to use less stringent conditions since we made the assumption that MA lines, on average, would be less stress tolerant and if all worms die we cannot test our hypotheses. The N2 lab strain has appeared to lose much of its thermotolerance compared to wild strains (Anderson *et al.* 2011 and references therein), but can survive for more than 10 hours in temperatures ranging up to 37°C. We modeled our thermotolerance assay on those of Lithgow and colleagues, (Lithgow *et al.* 1994; Lithgow *et al.* 1995); we exposed the nematodes to 35°C conditions because this temperature was likely to kill nematodes within a reasonably short period of time (Lithgow *et al.* 1994; Lithgow *et al.* 1995) while still allowing us to detect differences in thermotolerance in between our sets of nematodes. Inducing oxidative stress in nematodes (and, in fact, many other organisms) by exposing them to paraquat rapidly is becoming the standard approach in oxidative stress studies, with most authors adopting the methodology of Ishii and coworkers

(1990). Investigators tend to expose nematodes to paraquat concentrations ranging from 0.001 mM to 5 mM (e.g. Ishii *et al.* 1990; Yanase *et al.* 2002), we found 0.05 mM to be most effective. We used 0.05 mM of paraquat because it still allowed the nematodes to survive long enough for us to collect data, but had a significant effect on development. Our final stressor was high salt, which has not been tested as extensively as heat and paraquat. We based our salt concentration off of the Solomon (2004) paper, in which they used 500 mM of additional salt to stress the nematodes. We found that an increase from 50 mM to 500 mM NaCl in the agar stressed the nematodes too much and all of the nematodes died within 24 hours, regardless of strain or fitness category. Thus, we reduced our salt concentration to 300 mM, which was more effective at separating nematodes based on fitness category.

Experiment 1

Even though the stress treatments were effective enough that we could, typically, distinguish between those nematodes exposed to stress and those in control conditions, the stress treatments did not help to parse apart nematodes of similar fitness, such as comparisons within N2 and within HK104. As discussed above, the current characterization of base substitutions in N2 and HK104 suggest mutation accumulation in the two strains does not result in strain-specific patterns of base substitutions (rate nor substitution type)(Denver *et al.* 2012, in review); and yet, mutational declines in fitness were three-fold higher in HK104 than in N2 (Baer *et al.* 2005). Our work, like Baer's work, suggests that the strains are actually quite different and respond very differently to MA and to stress. Consistent with previous work (Matsuba *et al.* 2012, in review; Prasad

et al. 2011), the HK104 strain was considerably more thermotolerant in our heat stress assay than were N2 nematodes that were assayed concurrently.

In contrast, the HK104 MA lines performed significantly worse in the paraquat and salt assays than did both the N2 AC and MA lines, when compared within and across experiments. HK104 consistently performs 'worse' than N2, as evidenced by mutational declines in body size (Ostrow *et al.* 2007) and lifespan (Joyner-Matos *et al.* 2009), which is typically attributed to the greater mutational declines in fitness in HK104 (Baer *et al.* 2005). However, it also is possible that the HK104 MA lines may have been more susceptible to paraquat-induced oxidative stress because the HK104 AC and its derived MA lines have large, heteroplasmic deletions in the mitochondrial DNA (Howe *et al.* 2009). Since the large deletions are within the gene coding for subunit 5 of Complex I of the mitochondrial electron transport chain, the subunit that makes the greatest contribution to mitochondrial free radical production (for review, Halliwell and Gutteridge 1998), it is possible that HK104 has greater susceptibility to oxidative stress. Organisms that have high rates of mitochondrial free radical production tend to be more susceptible to exogenous oxidative stress, as demonstrated by the increased paraquat sensitivity of the *mev-1* mutant strain of N2 (has very high rates of mitochondrial free radical production) to the 'wild type' N2 (e.g., Ishii *et al.* 1990). It is possible that the increased paraquat susceptibility in HK104 that we detected in our assay results, in part, from consequences of the mitochondrial deletions. Additionally, there appears to be a higher rate of mitochondrial polymorphisms in HK104 than in N2 MA lines (Cutter *et al.* 2006; Estes *et al.* 2011), which may contribute to a heightened susceptibility to exogenous oxidative stress. The HK104 MA lines responded very similarly to high salt

stress as they did to paraquat. For example, MA line 261 was the most susceptible (survival and development) to both stress types while MA line 263 was the most robust and, across all lines, HK104 was more susceptible to paraquat and salt than were the concurrently-run N2 AC. This pattern also may reflect the apparent linkage between the cellular stress responses to exogenous oxidative stress and to hyperosmotic stress (Kültz 2004; Silva *et al.* 2005; Zhang *et al.* 2004). Since hyperosmotic stress tends to negatively impact the balance between free radical production and repair of free radical damage, as indicated by the accumulation of protein and DNA oxidative damage in cells exposed to high salt (Kültz 2004; Silva *et al.* 2005; Zhang *et al.* 2004), the 'balancing act' inherent to free radical metabolism may be less robust in HK104 (lower mitochondrial genome quality) than in N2.

In both blocks of experiment 1, we did not detect a significant effect of MA treatment (MA versus AC), which is considered a universal characteristic of MA experiments. However, this expectation of significant differences between MA and unmutated AC is perhaps untenable. For example, one of the early, foundational *C. elegans* MA papers (Lynch *et al.* 1999), failed to detect consistent significant differences in several fitness-related traits in N2. In Vassilieva (2000) and in our experiment 1, as discussed above, substantial within-line variance undoubtedly contributed to the lack of significant effect of MA treatment. An additional complication, which is only recently getting discussed in the literature, is the uncertainty as to how the assay environment may (or may not) influence the relative differences in trait means between MA and AC. For example, fitness-related traits in *Arabadopsis thaliana* MA lines were assayed under benign greenhouse (=standard growing and assaying) conditions and in (stressful) field

conditions (Rutter *et al.* 2009). Overall performance was lower in the field conditions, which is expected since plants growing in unmanaged fields experience a wide spectrum of factors that are not present in rigidly controlled greenhouse conditions. The surprising result was that the consistent pattern of greater fitness in unmutated AC than in MA lines that was exhibited in the greenhouse disappeared in the field. In fact, in the field, the AC was indistinguishable from the trait mean of the MA lines (literally, half of the MA lines had higher fitness than the AC, half had lower). The authors speculated that the unusually high number of MA lines that outperformed the unmutated ancestor indicated the presence of beneficial mutations that aide in responses to environmental stressors that are not present in benign, greenhouse conditions, an interpretation that is not universally supported (Baer, pers. comm.). The degree to which this interpretation of the finding that AC and MA lines were indistinguishable when assayed in stressful conditions applies to our work is unclear. However, ongoing statistical analyses of the second-order MA lines will elucidate the impact of beneficial mutations on the second-order fitness results; when that analysis is complete, it would be interesting to compare the degree to which this pattern (MA and AC indistinguishable in stress but not control conditions) exists in the sequenced line data versus the second order data.

Here we suggest, for the first time (to our knowledge), that our inability to distinguish MA from AC also could be due to the transcriptional freedom of the ancestor as compared to that of the MA lines. Since nematodes in the wild are exposed to rapidly changing environments, it is beneficial to have a wide range of cellular pathways and underlying gene expression plasticity to respond to fluctuating stressors. Since the N2 AC has been in the lab setting for decades, it has lost some of its ability to quickly and

appropriately respond to stress (for review, Gershon and Gershon 2002). In the typical MA paradigm, one would predict that plasticity, or sensitivity of gene expression in the presence of a fluctuating environment would be even lower in MA lines than in the unmutated ancestor since accumulated mutations presumably impact the regulation of gene expression and the quality of gene transcripts. However, Baer and Denver (2010) found the opposite pattern. In their study of N2 AC and MA lines, transcript abundance and diversity was higher in N2 MA lines than in the N2 AC. We speculate that this apparent constraint on cellular responsiveness in N2 AC may contribute to similar stress susceptibilities of AC and MA nematodes in our assays. Perhaps the AC nematodes have converged on a weak response to stress because that is the only option available to them given their limited transcriptional flexibility. Across MA lines, in contrast, some MA lines have more (than AC) robust stress responses, while others have less robust responses, which may reflect varying levels of transcriptional flexibility. The MA lines in our assays are not the same as those used in the transcription study, so this concept remains speculative at this point. However, when taken in combination with the *Arabidopsis* greenhouse versus field results and the within-line variance, it is not surprising that we did not detect a significant effect of MA treatment in experiment 1.

While we could not accurately predict base substitution rates from physiological robustness, there were interesting trends to be found in comparing individual MA lines. Within the N2 sequenced MA lines, certain lines tended to respond worse to stress than other MA lines, a pattern that tended to be consistent across stress type. Specifically, in all but the heat assay, MA line 523 had the worse survival and development compared to the other MA lines. The poor performance of MA line 523 is not reflected in the current

base substitution rates (Denver *et al.* 2012, in review) but is supported by its performance in other types of assays, including total oxidative damage (Joyner-Matos, pers. comm.) and reactive oxygen species (ROS; Estes, pers. comm.). The lines that did the 'best' varied between assays and no single line did the best. While annotation information (gene identification and coding versus noncoding sequence) is not available for the base substitutions identified in Denver's 2012 sequencing paper, this information is available for the 2009 paper. Unfortunately, a quick survey of the gene identities for all base substitutions that occurred in protein-coding sequences only helps explain some trends. For example, MA line 545 had mutations in coding regions of three genes that directly involved in heat shock responses and this line was least thermotolerant of the MA lines. On the other hand, as far as we can assess with the genome annotation to date, none of the genes impacted by mutations in MA line 523 have a clear link to oxidative stress and only one gene is tentatively linked to "embryo osmotic integrity" (www.wormbook.org). Another potential explanation is that a (or a few) mutation in 523 exhibits global pleiotropy (one gene influencing multiple phenotypic traits) across paraquat- and salt-related tolerance traits. In other words, it is possible that one or a few mutations that occurred in 523 might have very large (pleiotropic) effects while the number of base substitutions is lower than that of other MA lines and the set of mutations in this lineage (not yet characterized) collectively have a relatively small effect on fitness.

Experiment 2

As discussed above, we were more successful in detecting differences in physiological robustness across fitness categories than we were across sequenced lines. Across all assays in experiment 2, the low fitness MA lines consistently performed the

worst in comparison to the ancestor and high fitness MA lines. Differences in performance between the ancestor and high MA lines were somewhat more variable across the stress assays, perhaps reflecting differences in transcriptional sensitivity and/or the presence of beneficial mutations. Within the paraquat assay, nematodes from the high MA lines were the most robust, but were generally more similar to AC nematodes in the salt assay and were highly variable in the heat assay. Our inferences about high fitness MA versus AC are somewhat hindered by the large block effect, and we base our interpretations below more on ΔM values because they are calculated using the respective block ancestor. The low fitness MA lines may have performed poorly because of (potentially) restricted transcriptional freedom, overall lower quality genome, or pleiotropy. The ancestor and high MA lines performed worse than the low MA lines in some of the traits but this is partly attributable to the block effect and may reflect transcriptional constraints in AC.

The relationship between physiological robustness and fitness category may have been stronger if not for the large block effect (Table 5). The block effect may have occurred because of picking variation in pickers and time, variation in data recording, fluctuations in chemical concentrations or heat variations, or other possibilities. Block effects are rather prevalent in MA experiments and relatively under-emphasized in the older MA literature (Baer, pers. comm.). What we do know is that there was a clear block effect on survival and development in the paraquat and salt assays, especially in block 2, in which all nematodes had significantly lower survival and development than block 1. Interestingly, the block effect appeared to be the most dramatic for the ancestor and intermediate for the high MA lines in the paraquat and salt assays. The ancestor had no

particular pattern across traits and blocks; in some traits, the ancestor did the best in block 1 and worst in block 2, and in other traits the pattern was opposite. A similar messiness existed in the high MA line dataset, but to a lesser extent. This variability across blocks was much less pronounced in the low fitness MA lines, perhaps because the low fitness MA lines performed so poorly overall that they could not do much worse and were unlikely to improve.

The motility and body size assays were intended to serve as preliminary experiments to determine whether these traits might be mutational targets (i.e., decline in MA lines) and merit further investigation. As noted above, nematode body size (Ostrow *et al.* 2007) and chemotactic behaviors (Ajie *et al.* 2005; Estes *et al.* 2005) decline with mutation accumulation. With exception of altering plate type (addition of paraquat/salt), our body size assay was comparable to Ostrow and coworkers (2007). This is not the case for our motility assay. In the chemotaxis, or locomotion studies, nematodes were cultured in standard lab conditions and exposed to a repellant chemical; MA nematodes were significantly slower and were more directionally-impaired than were AC nematodes (Ajie *et al.* 2005; Estes *et al.* 2005). However, these studies did not discriminate between mutational effects on chemosensation (sensing the cue) and mutational effects on whole-organism motility and coordination (responding to the cue). Since Ajie and Estes and their coworkers demonstrated that, to some degree, motility is a mutational target, we decided to assay motility. We assayed motility under standard conditions (as they did) and with the added component of exogenous stress since behaviors are often impacted by stress. We incorporated environmental stress factors (paraquat or salt) with the motility assay. We tested for differences across fitness categories in steady-state movement (no

chemoattractant nor repellant), with and without environmental stress. Secondarily, we wanted to know if the stress treatments were severe enough to cause changes in locomotion and morphology. We were surprised to find that the motility and body size assay, even with the low sample size, was sensitive enough to detect an effect of stress treatment in the AC and MA lines. The motility and body size assays are worth the time to do only if they are run alongside additional assays and direct (within line replicate) comparisons can be made. By themselves, they were not sensitive enough to make any definitive claims about mutational declines in motility/body size or mutational declines in stress susceptibility, but taken with the other stress assays, it helps give the whole picture of what is occurring.

The heat assay in experiment 2 was the least informative assay in terms of distinguishing between fitness categories because there was a large block effect from the block 2 results. In block 2, all of the nematodes had very low survival compared to any of the nematodes in block 1. In block 1, the AC and MA lines performed like we expected. The AC line had the highest mean survival, the high MA lines had intermediate survival, and the low MA lines had the lowest survival. In block 1, physiological response correlated with fitness category but with block 2 added, there was no correlation between the two.

The paraquat assay in experiment 2 was far more convoluted than the heat assay. The low fitness MA lines tended to have the poorest performance (survival and development) across blocks, but low fitness MA lines usually were not significantly different from the other categories because of the block effect. The AC and high fitness MA lines generally did better than low fitness lines, but sometimes had the worst

performance in a given trait (see Figures 22, 23, 25, and 34). The most informative traits for distinguishing among fitness categories were the proportion of adults present, overall survival, development score, and motility. Traits that did not have a consistent pattern were survival to a specific day, days to reach adulthood, and body size. Much like the heat assay, the paraquat assay also had a significant block effect. When looking at all of the traits as a whole, though, it was obvious that nematodes from the low fitness MA lines had lower survival and slower development over the study period. Nematodes from the AC and high fitness MA lines generally had similar survival and development, but in some of the traits the ancestor did best and in other traits, the high MA lines did best. The reason nematodes from the low fitness MA lines performed poorly could be due to large mutational declines and, potentially, smaller transcriptional freedom. It is unclear why the nematodes from the ancestor and high fitness MA lines performed similarly, but see our discussion of this point above (for experiment 1 MA versus AC comparisons). The motility assay generated rather unexpected results in the paraquat experiment. To our knowledge, heightened activity in the presence of exogenous oxidative stress has not been documented. On NGM, ancestors had greater motility than did MA nematodes (See Table 12), but on paraquat plates, MA nematodes were significantly more active, and nematodes from high fitness lines were the most active. We cannot speculate as to why activity would higher paraquat than on other plate types, or why activity would decrease after 48 hours on paraquat, but this is clearly a very interesting avenue for future research. The consequences of paraquat exposure on body size are less clear, but do seem to vary across fitness category and could be informative if assayed at greater sample size. Overall, these paraquat assay results suggest that if we were able to replicate this

experiment and assay all 10 MA lines at the same time, we would demonstrate that tolerance of exogenous oxidative stress is a sensitive enough metric to discriminate between groups of lines that differ significantly by fitness.

The salt assay from experiment 2 had somewhat similar results to the paraquat assay. Nematodes from the low fitness MA lines generally had lower survival and slower development than did nematodes from the ancestor and high fitness MA lines. There was also a significant block effect that occurred across all traits and again, in block 1, nematode survival tended to be higher and development tended to be faster. The main difference between the paraquat and salt assays were the performances of the high fitness MA lines. Nematodes from the high fitness MA lines generally had the highest survival and fastest development across traits and blocks in the salt assay. This is curious, given how similar the ancestor and high fitness MA lines were in the paraquat assay. While it is clear that at least some of the cellular-level response to hyperosmotic stress involves responding to oxidative stress, there is clearly much more to hyperosmotic shock than just increased oxidative damage. In comparison to the paraquat assay, nematode motility on salt plates was similar to that of nematodes on NGM, but there are some indications in this preliminary study that nematodes from low fitness lines move less when on salt than do nematodes from the other categories. Proportional changes in body size (over time) were greatest in nematodes on salt plates, but the block effect obscures any differences between fitness categories.

As mentioned above, Baer has re-assayed one of the second order MA lines and is in the process of revising his second-order manuscript. Since the data are considered, “in revision” and not, “in review” we only can make qualitative comparisons between our

stress metrics and the mutational declines in fitness between his MA generations 250 and 375 (the "second-order" MA). Whether analyzed with the aberrant second-order MA line or without it, Baer's data set supports his hypothesis. Mutational declines in fitness were greater in the low fitness MA lines than in the high fitness MA lines, suggesting that genome quality affects future mutation rate. When the aberrant MA line was removed from the data set, mean mutational declines in fitness were nearly 2.5 times higher in the low fitness MA lines than in the high fitness MA lines (statistically significant without the aberrant line). Preliminary reports from Baer indicate that this result holds up with the re-assay of the one line, but statistical analyses are not complete. Ranking his second-order fitness results by line mean, the greatest mutational declines in fitness among the five "low fitness MA lines" were in MA lines 508 and 504 and the smallest decline in fitness was in MA line 550 (mutational decline in fitness: 508 < 504 < 547 < 550). Overall, our block 1 results tend to support this trend, which would, at a very preliminary level, suggest that stress susceptibility may be predictive of future mutation rate. However, as usual, this trend does not hold in block 2 and our overall strength of inference is weak. Among the "high fitness MA lines," mutational declines in fitness (Baer's assay) were lowest in MA line 583 and highest in MA line 522 (mutational decline in fitness; 583 < 566 < 587 < 537 < 522) and, as in the other fitness category, our block 1 results are similar to Baer's results but our block 2 data are not. Overall, our preliminary analysis of the potential relationship between stress susceptibility and future mutation rate only takes into account line means and not within-line variance; a complete test of this idea should include both correlations of line means and covariation between

stress and mutation rate. Once Baer's statistical analysis is complete, we will be able to assess covariation between traits.

Finally, despite the block effect, the experiment 2 data indicate that we perhaps should be thinking of genome quality as a source of stress, and that when nematodes with low quality genomes are exposed to an additional, exogenous stress, they have a blunted ability to respond to the environmental stress. This pattern is more apparent in experiment 2 than in experiment 1, likely because fitness differences between lines are much greater in the lines assayed in experiment 2 than in experiment 1. These results support Agrawal's hypothesis concerning the potential interdependence between condition and mutation rate (Agrawal 2002) since these results suggest that the inability to robustly respond to environmental stress may indicate decreased resources for mutation detection and repair and thus a heightened likelihood of passing mutations on to offspring.

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APPENDIX A: TABLES, FIGURE LEGENDS, AND FIGURES

Tables are presented first, with the caption present above each table. Figure legends are presented separately from the figures. Each figure is accompanied by a short, descriptive title (identical to that in the figure legend) to aide the reader in identifying figures.

Table 1: Definitions of traits measured in heat, paraquat, and salt assays and brief descriptions of how they were calculated. The XX indicate assay-specific numbers of hours or numbers of days.

Assay Type	Trait	Definition and Calculation
Heat	Survival at XX hours in heat stress (35°C) or heat control (20°C)	Total number of nematodes alive at XX hours, calculated as a proportion of the ten nematodes put on each plate
Paraquat or Salt	Survival to XX days	Total number of nematodes alive on XX day, calculated as a proportion, out of the ten nematodes that were put (as eggs) on each plate (paraquat assay) or the five nematodes that were put (as eggs) on the agar in each microwell (salt assay)
	Proportion adults on day XX	Proportion of hatched nematodes that developed to adulthood by day XX
	Number of days to adulthood	The number of days until the first adult nematodes were found on each plate/well
	Survival to adulthood	The proportion of hatched nematodes that ever developed to adulthood
	Development score	Combined metric of survival and development. Nematodes in each plate/well were classified into developmental stages, the number of nematodes in each stage was multiplied by a stage-specific value (L1 larvae = 1, L2 larvae = 2, L3 larvae = 3, L4 larvae = 4, adult = 5) and summed. For example, a plate with 8 adults and 2 L4 nematodes would have the score calculated as: 8*5 + 2*4 = 48. Maximum possible score in the paraquat assay (10 nematodes /plate) was 50; maximum possible score in the salt assay (5 nematodes /plate) was 25.
	Decrement in development score	Indicates the relative difference between development score in control conditions (NGM) versus stress conditions (paraquat or salt); calculated as: (score on NGM – score on stress)/score on NGM. Decrements were calculated with line means.
All assays	delta M (ΔM)	Mutational change in a trait; calculated as $(Mean_{MA} - Mean_{AC})/(Mean_{AC}*250)$

Table 2: Experiment 1, proportion of nematodes that survived to each time point (in hours, H) in control conditions (20°C) and heat stress (35°C). Data are presented as mean (standard error). Abbreviations: AC, ancestral control; MA, mutation accumulation; N2 and HK104 indicate strains of *C. elegans* and *C. briggsae*, respectively. The N2 AC was present in both assays. Blank cells indicate that nematode survival was not measured at that time point.

Strain	MA vs. AC	Line	18/22 H Control	18/19 H Heat	22 H Heat	24 H Heat	27 H Heat	29 H Heat	40 H Heat
N2	AC	AC	0.98 (0.02)	0.15 (0.1)	0.07 (0.1)	--	0.05 (0.1)	--	--
	MA	523	0.98 (0.02)	0.75 (0.1)	0.40 (0.1)	--	0.25 (0.1)	--	--
	MA	526	0.94 (0.02)	0.67 (0.1)	0.55 (0.2)	--	0.44 (0.2)	--	--
	MA	529	1.00 (0.0)	0.56 (0.1)	0.42 (0.1)	--	0.26 (0.1)	--	--
	MA	545	1.00 (0.0)	0.39 (0.2)	0.14 (0.1)	--	0.12 (0.1)	--	--
	MA	553	1.00 (0.0)	0.85 (0.1)	0.73 (0.1)	--	0.53 (0.1)	--	--
	MA	574	0.86 (0.1)	0.76 (0.1)	0.48 (0.1)	--	0.34 (0.1)	--	--
HK104	AC	AC	0.98 (0.02)	0.94 (0.03)	0.87 (0.04)	0.86 (0.04)	0.85 (0.1)	0.72 (0.1)	0.31 (0.05)
	MA	232	0.80 (0.03)	0.93 (0.02)	0.86 (0.05)	0.82 (0.03)	0.79 (0.03)	0.65 (0.1)	0.33 (0.1)
	MA	261	0.98 (0.02)	0.82 (0.07)	0.71 (0.1)	0.54 (0.1)	0.41 (0.1)	0.20 (0.1)	0.05 (0.03)
	MA	263	0.84 (0.1)	0.92 (0.03)	0.84 (0.04)	0.83 (0.1)	0.81 (0.1)	0.66 (0.1)	0.33 (0.03)
N2	AC	AC	1.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)

Table 3: Experiment 1, proportion of nematodes that survived to each day in control conditions (nematode growth medium) and on paraquat-containing plates. Data are presented as mean (standard error). Abbreviations: AC, ancestral control; MA, mutation accumulation; N2 and HK104 indicate strains of *C. elegans* and *C. briggsae*, respectively. The N2 AC was present in both assays. Blank cells indicate that nematode survival was not measured at that time point.

Strain	MA vs. AC	Line	Day 2 Control	Day 2 Paraquat	Day 3 Control	Day 3 Paraquat	Day 4 Paraquat	Day 5 Paraquat	Day 6 Paraquat
N2	AC	AC	0.82 (0.01)	0.63 (0.2)	0.80 (0.1)	0.54 (0.1)	0.82 (0.1)	0.51 (0.04)	0.17 (0.1)
	MA	523	0.82 (0.1)	0.72 (0.1)	0.92 (0.04)	0.53 (0.05)	0.39 (0.1)	0.39 (0.1)	0.27 (0.1)
	MA	526	0.80 (0.1)	0.80 (0.1)	0.94 (0.04)	0.63 (0.1)	0.60 (0.1)	0.67 (0.1)	0.53 (0.1)
	MA	529	0.74 (0.1)	0.82 (0.04)	0.80 (0.1)	0.63 (0.1)	0.62 (0.04)	0.63 (0.03)	0.33 (0.2)
	MA	545	0.76 (0.1)	0.68 (0.1)	0.74 (0.1)	0.62 (0.1)	0.50 (0.1)	0.56 (0.1)	0.31 (0.2)
	MA	553	0.88 (0.04)	0.89 (0.03)	0.90 (0.1)	0.83 (0.03)	0.81 (0.04)	0.81 (0.1)	0.41 (0.1)
	MA	574	0.66 (0.2)	0.86 (0.1)	0.72 (0.2)	0.60 (0.1)	0.70 (0.1)	0.70 (0.05)	0.63 (0.1)
HK104	AC	AC	0.84 (0.2)	0.72 (0.1)	0.84 (0.1)	0.66 (0.1)	0.68 (0.1)	0.63 (0.1)	0.21 (0.1)
	MA	232	0.64 (0.1)	0.62 (0.05)	0.62 (0.1)	0.58 (0.1)	0.55 (0.1)	0.49 (0.1)	0.31 (0.1)
	MA	261	0.58 (0.1)	0.44 (0.1)	0.66 (0.1)	0.45 (0.1)	0.42 (0.1)	0.38 (0.1)	0.19 (0.1)
	MA	263	0.88 (0.1)	0.71 (0.1)	0.86 (0.1)	0.72 (0.1)	0.62 (0.1)	0.57 (0.1)	0.31 (0.1)
N2	AC	AC	1.00 (0.0)	0.80 (0.0)	1.00 (0.0)	0.70 (0.1)	0.70 (0.1)	0.50 (0.1)	--

Table 4: Experiment 1, proportion of nematodes that survived to each day in control conditions (nematode growth medium) and on high salt plates. Data are presented as mean (standard error). Abbreviations: AC, ancestral control; MA, mutation accumulation; N2 and HK104 indicate strains of *C. elegans* and *C. briggsae*, respectively. The N2 AC was present in both assays.

Strain	MA vs. AC	Line	Day 2 Control	Day 2 Salt	Day 3 Control	Day 3 Salt	Day 4 Salt	Day 5 Salt	Day 6 Salt
N2	AC	AC	0.76 (0.1)	0.53 (0.1)	0.90 (0.04)	0.53 (0.1)	0.59 (0.1)	0.50 (0.1)	0.05 (0.04)
	MA	523	0.84 (0.04)	0.21 (0.1)	0.9 (0.03)	0.33 (0.05)	0.32 (0.1)	0.39 (0.1)	0.17 (0.1)
	MA	526	0.58 (0.1)	0.50 (0.1)	0.56 (0.1)	0.60 (0.1)	0.59 (0.1)	0.67 (0.1)	0.05 (0.04)
	MA	529	0.48 (0.1)	0.51 (0.03)	0.50 (0.1)	0.47 (0.1)	0.34 (0.04)	0.63 (0.03)	0.12 (0.1)
	MA	545	0.68 (0.04)	0.45 (0.04)	0.74 (0.1)	0.50 (0.1)	0.46 (0.1)	0.56 (0.1)	0.12 (0.1)
	MA	553	0.80 (0.1)	0.39 (0.1)	0.80 (0.1)	0.34 (0.1)	0.35 (0.1)	0.81 (0.1)	0.13 (0.05)
	MA	574	0.84 (0.1)	0.43 (0.1)	0.88 (0.1)	0.50 (0.1)	0.46 (0.1)	0.70 (0.05)	0.09 (0.1)
HK104	AC	AC	0.60 (0.2)	0.09 (0.03)	0.60 (0.2)	0.07 (0.03)	0.09 (0.05)	0.08 (0.04)	0.07 (0.04)
	MA	232	0.22 (0.1)	0.33 (0.1)	0.16 (0.1)	0.33 (0.1)	0.26 (0.1)	0.21 (0.04)	0.15 (0.04)
	MA	261	0.46 (0.1)	0.06 (0.03)	0.42 (0.1)	0.09 (0.03)	0.08 (0.03)	0.07 (0.03)	0.07 (0.03)
	MA	263	0.34 (0.1)	0.34 (0.1)	0.42 (0.1)	0.35 (0.1)	0.36 (0.1)	0.29 (0.04)	0.22 (0.1)
N2	AC	AC	1.00 (0.0)	0.40 (0.1)	1.00 (0.0)	0.50 (0.1)	0.50 (0.1)	0.50 (0.1)	0.10 (0.0)

Table 5: Experiment 2, metrics of survival and development in the ancestral control (AC) of the N2 strain of *C. elegans* across the two assay blocks. Traits are defined in Table 1. Data are presented as mean (standard error).

Block	Fitness category	Line	Heat Control	Survival to Adulthood Paraquat	Survival to Adulthood Salt	Number of Days to Adulthood Paraquat	Number of Days to Adulthood Salt	Proportion Adults on Day 5 Paraquat	Proportion Adults on Day 5 Salt
1	AC	AC	0.92 (0.1)	0.67 (0.1)	0.10 (0.03)	5.6 (0.4)	6.0 (0.3)	0.58 (0.2)	0.04 (0.04)
2	AC	AC	0.92 (0.1)	0.15 (0.1)	0.26 (0.1)	6.9 (0.5)	5.7 (0.2)	0.02 (0.02)	0.28 (0.1)

Table 6: Experiment 2, proportion of nematodes from the N2 strain of *C. elegans* that survived to each time point (in hours, H) in control conditions (20°C) and heat stress (35°C). Data are presented as mean (standard error). Abbreviations: AC, ancestral control; MA, mutation accumulation; High MA, high fitness MA line; Low MA, low fitness MA line; ΔM (x 10^3), mutational change in the trait mean, calculated as percent change per MA generation. The N2 AC was present in both assays.

Block	Fitness category	Line	18 H Control	18 H Heat	23 H Heat	25 H Heat
1	AC	AC	0.92 (0.1)	0.85 (0.1)	0.083 (0.1)	0.08 (0.1)
	High MA	522	0.92 (0.1)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)
	High MA	537	0.94 (0.04)	0.27 (0.1)	0.02 (0.01)	0.01 (0.01)
	High MA	566	0.90 (0.03)	0.86 (0.0)	0.57 (0.1)	0.50 (0.1)
	Low MA	504	0.80 (0.1)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)
	Low MA	547	0.82 (0.1)	0.50 (0.1)	0.09 (0.04)	0.07 (0.03)
	High MA	ΔM (x 10^3)	0.000	-2.227	-3.052	-3.171
	Low MA	ΔM (x 10^3)	0.239	-2.824	-3.783	-3.829
2	AC	AC	0.92 (0.1)	0.38 (0.1)	--	--
	High MA	583	0.95 (0.1)	0.35 (0.2)	--	--
	High MA	587	1.00 (0.0)	0.34 (0.1)	--	--
	Low MA	508	0.98 (0.03)	0.80 (0.1)	--	--
	Low MA	550	0.80 (0.1)	0.09 (0.1)	--	--
	Low MA	579	0.97 (0.1)	0.43 (0.1)	--	--
	High MA	ΔM (x 10^3)	-0.478	-0.368	--	--
	Low MA	ΔM (x 10^3)	-0.022	0.614	--	--
1 & 2	High MA	ΔM (x 10^3)	0.096	-1.484	--	--
	Low MA	ΔM (x 10^3)	-0.204	-0.761	--	--

Table 7: Experiment 2, proportion of nematodes from the N2 strain of *C. elegans* that survived to each time point (in hours, H) in control conditions (nematode growth medium) and on paraquat-containing plates. Data are presented as mean (standard error). Abbreviations: AC, ancestral control; MA, mutation accumulation; High MA, high fitness MA line; Low MA, low fitness MA line; ΔM (x 10^3), mutational change in the trait mean, calculated as percent change per MA generation. The N2 AC was present in both assays.

Block	Fitness category	Line	Day 2 Control	Day 2 Paraquat	Day 3 Control	Day 3 Paraquat	Day 4 Paraquat	Day 5 Paraquat	Day 6 Paraquat
1	AC	AC	0.92 (0.04)	0.96 (0.03)	0.96 (0.04)	0.82 (0.1)	0.81 (0.1)	0.85 (0.04)	0.30 (0.2)
	High MA	522	0.96 (0.0)	0.84 (0.03)	0.94 (0.04)	0.63 (0.1)	0.59 (0.04)	0.60 (0.0)	0.13 (0.1)
	High MA	537	0.84 (0.04)	0.81 (0.1)	0.90 (0.1)	0.63 (0.1)	0.63 (0.04)	0.61 (0.03)	0.62 (0.04)
	High MA	566	0.72 (0.1)	0.90 (0.1)	0.86 (0.1)	0.77 (0.05)	0.77 (0.0)	0.69 (0.04)	0.12 (0.1)
	Low MA	504	0.64 (0.1)	0.76 (0.1)	0.72 (0.1)	0.48 (0.1)	0.59 (0.04)	0.45 (0.04)	0.40 (0.1)
	Low MA	547	0.60 (0.1)	0.76 (0.1)	0.56 (0.1)	0.63 (0.0)	0.60 (0.1)	0.55 (0.1)	0.21 (0.1)
	High MA	ΔM (x 10^3)	--	--	-0.222	-0.699	-0.724	-1.020	--
	Low MA	ΔM (x 10^3)	--	--	-1.333	-1.293	-1.062	-1.647	--
2	AC	AC	0.28 (0.1)	0.30 (0.1)	0.32 (0.1)	0.19 (0.05)	0.19 (0.04)	0.21 (0.1)	0.20 (0.1)
	High MA	583	0.30 (0.1)	0.21 (0.1)	0.44 (0.1)	0.17 (0.1)	0.26 (0.1)	0.32 (0.2)	0.28 (0.1)
	High MA	587	0.62 (0.1)	0.58 (0.1)	0.62 (0.1)	0.24 (0.04)	0.36 (0.1)	0.41 (0.1)	0.37 (0.1)
	Low MA	508	0.28 (0.1)	0.37 (0.1)	0.34 (0.1)	0.39 (0.1)	0.30 (0.1)	0.28 (0.1)	0.20 (0.1)
	Low MA	550	0.22 (0.1)	0.27 (0.1)	0.22 (0.1)	0.24 (0.1)	0.25 (0.1)	0.40 (0.1)	0.33 (0.1)
	Low MA	579	0.28 (0.1)	0.37 (0.1)	0.28 (0.1)	0.35 (0.1)	0.35 (0.1)	0.33 (0.1)	0.22 (0.1)
	High MA	ΔM (x 10^3)	--	--	0.930	0.316	2.526	1.345	--
	Low MA	ΔM (x 10^3)	--	--	-1.395	2.877	2.316	-0.085	--
1 & 2	High MA	ΔM (x 10^3)	--	--	0.239	-0.293	0.576	-0.074	--
	Low MA	ΔM (x 10^3)	--	--	-1.371	1.209	0.965	-0.710	--

Table 8: Experiment 2, ΔM values of developmental traits of nematodes in the paraquat assay. Fitness categories are: AC, ancestral control; High MA, high fitness MA lines; Low MA, low fitness MA lines. ΔM values were calculated for select days as described in the Materials and Methods and in Table 1 for each block separately and then averaged across block. Traits are defined in Table 1.

Block	Fitness category	Line	Survival to Adulthood Control	Survival to Adulthood Paraquat	Number of days to Adulthood Control	Number of days to Adulthood Paraquat	Proportion Adults on day 5 Paraquat
1	High MA	ΔM (x 10^3)	0.000	-2.070	0.000	0.013	-1.591
	Low MA	ΔM (x 10^3)	-1.343	-3.143	0.000	0.429	-3.091
2	High MA	ΔM (x 10^3)	1.900	-0.125	0.000	-0.336	17.712
	Low MA	ΔM (x 10^3)	-1.833	-2.333	0.000	-0.465	11.997
1 & 2	High MA	ΔM (x 10^3)	0.760	-1.292	0.000	-0.127	6.130
	Low MA	ΔM (x 10^3)	-1.637	-2.654	0.000	-0.108	5.962

Table 9: Experiment 2, proportion of nematodes from the N2 strain of *C. elegans* that survived to each time point (in hours, H) in control conditions (nematode growth medium) and on high salt agar. Data are presented as mean (standard error). Abbreviations: AC, ancestral control; MA, mutation accumulation; High MA, high fitness MA line; Low MA, low fitness MA line; ΔM (x 10^3), mutational change in the trait mean, calculated as percent change per MA generation. The N2 AC was present in both assays.

Block	Fitness category	Line	Day 2 Control	Day 2 Salt	Day 3 Control	Day 3 Salt	Day 4 Salt	Day 5 Salt	Day 6 Salt
1	AC	AC	0.74 (0.1)	0.11 (0.0)	0.80 (0.1)	0.20 (0.1)	0.23 (0.1)	0.22 (0.1)	0.16 (0.1)
	High MA	522	0.84 (0.1)	0.37 (0.1)	0.90 (0.1)	0.43 (0.1)	0.50 (0.1)	0.28 (0.1)	0.12 (0.1)
	High MA	537	0.98 (0.02)	0.37 (0.1)	0.96 (0.04)	0.50 (0.1)	0.54 (0.1)	0.15 (0.1)	0.10 (0.1)
	High MA	566	0.76 (0.1)	0.37 (0.1)	0.80 (0.1)	0.44 (0.1)	0.44 (0.1)	0.40 (0.1)	0.22 (0.05)
	Low MA	504	0.64 (0.2)	0.03 (0.0)	0.82 (0.1)	0.05 (0.04)	0.07 (0.03)	0.08 (0.0)	0.06 (0.03)
	Low MA	547	0.58 (0.1)	0.35 (0.1)	0.64 (0.1)	0.35 (0.1)	0.38 (0.1)	0.31 (0.1)	0.24 (0.1)
	High MA	ΔM (x 10^3)	--	--	0.433	5.133	4.580	1.030	--
	Low MA	ΔM (x 10^3)	--	--	-0.350	0.000	-0.087	-0.455	--
2	AC	AC	0.36 (0.2)	0.24 (0.1)	0.30 (0.2)	0.23 (0.05)	0.29 (0.1)	0.30 (0.1)	0.21 (0.04)
	High MA	583	0.14 (0.1)	0.17 (0.1)	0.06 (0.04)	0.18 (0.04)	0.20 (0.1)	0.21 (0.1)	0.14 (0.0)
	High MA	587	0.16 (0.1)	0.29 (0.05)	0.22 (0.1)	0.28 (0.03)	0.30 (0.1)	0.36 (0.04)	0.21 (0.03)
	Low MA	508	0.20 (0.1)	0.28 (0.1)	0.22 (0.1)	0.19 (0.1)	0.10 (0.04)	0.03 (0.0)	0.01 (0.01)
	Low MA	550	0.14 (0.1)	0.07 (0.04)	0.22 (0.1)	0.11 (0.1)	0.15 (0.05)	0.16 (0.1)	0.10 (0.1)
	Low MA	579	0.28 (0.1)	0.09 (0.03)	0.20 (0.1)	0.16 (0.03)	0.20 (0.1)	0.15 (0.1)	0.06 (0.04)
	High MA	ΔM (x 10^3)	--	--	-2.133	0.000	-0.552	-0.200	--
	Low MA	ΔM (x 10^3)	--	--	-1.156	-1.333	-1.931	-2.489	--
1 & 2	High MA	ΔM (x 10^3)	--	--	-0.593	3.080	2.527	0.538	--
	Low MA	ΔM (x 10^3)	--	--	-0.833	-0.800	-1.193	-1.675	--

Table 10: Experiment 2, ΔM values of developmental traits of nematodes in the salt assay. Fitness categories are: AC, ancestral control; High MA, high fitness MA lines; Low MA, low fitness MA lines. ΔM values were calculated as described in the Materials and Methods and in Table 1 for each block separately and then averaged across block. Traits are defined in Table 1.

Block	Fitness category	Line	Survival to adulthood Control	Survival to adulthood Salt	Number of days to adulthood Control	Number of days to adulthood Salt	Proportion adults on day 5 Salt
1	High MA	ΔM (x 10^3)	1.481	5.067	0.000	-0.421	4.133
	Low MA	ΔM (x 10^3)	-0.593	-1.400	0.000	0.655	-2.800
2	High MA	ΔM (x 10^3)	-1.600	-1.154	0.000	-0.050	-0.485
	Low MA	ΔM (x 10^3)	-1.689	-3.128	0.000	0.072	-3.232
1 & 2	High MA	ΔM (x 10^3)	0.249	2.578	0.000	-0.273	2.286
	Low MA	ΔM (x 10^3)	-1.250	-2.437	0.000	0.305	-3.060

Table 11: Experiment 2, ΔM values of proportional changes in body size from time 0 to 48 hours, averaged across MA line within fitness category and within plate type (control, nematode growth medium; paraquat-containing, and high salt plates). Fitness categories are: AC, ancestral control; High MA, high fitness MA lines; Low MA, low fitness MA lines. ΔM values were calculated as described in the Materials and Methods and in Table 1.

Block	Fitness category	ΔM (x 10^3) Control	ΔM (x 10^3) Paraquat	ΔM (x 10^3) Salt
1	High MA	-2.516	2.166	0.214
	Low MA	8.377	1.582	7.190
2	High MA	-0.492	-0.454	-0.502
	Low MA	-1.877	-0.360	-1.521
1 & 2	High MA	-1.707	1.118	-0.072
	Low MA	2.225	0.417	1.963

Table 12: Experiment 2, total motility of nematodes on control plates (nematode growth medium), paraquat-containing and high salt plates. Data are presented as mean (standard error) and indicate total number of grid boxes contacted by each nematode in a 10-second period and are averaged across mutation accumulation (MA) lines or ancestral control (AC) pseudolines and across block. Fitness categories are: AC, ancestral control; High MA, high fitness MA lines; Low MA, low fitness MA lines. Proportional changes in motility from time 0 to 48 hours were calculated (not shown), ΔM (x 10^3) values describe patterns in proportional changes in motility. ΔM values were calculated as described in the Materials and Methods and in Table 1.

Block	Fitness category	Trait	Control	Paraquat	Salt
1 & 2	AC	Time 0	3.98 (1.8)	10.5 (1.8)	3.5 (1.4)
	High MA	Time 0	5.08 (1.2)	12.1 (1.6)	2.3 (0.8)
	Low MA	Time 0	5.44 (1.6)	8.9 (2.4)	2.0 (0.8)
	AC	48 Hours	5.02 (1.4)	9.1 (2.1)	4.4 (1.1)
	High MA	48 Hours	3.79 (0.6)	7.9 (1.9)	5.0 (1.4)
	Low MA	48 Hours	3.96 (1.5)	6.5 (2.0)	3.1 (1.2)
	High MA	ΔM (x 10^3)	-8.94	7.95	8.16
	Low MA	ΔM (x 10^3)	-9.68	2.89	0.76

FIGURE LEGENDS

Figure 1. Mutation accumulation in *C. elegans*. A homozygous hermaphrodite is placed on a NGM plate and allowed to produce generation 1. A single individual is selected from this plate and allowed to lay the eggs that constitute generation 2. This continues for as long as the population remains viable. Spontaneous mutations, indicated here as a change from + to X, can be fixed in the lineage if the egg and/or sperm that combined to form the nematode that is randomly selected as the parent for the next generation contain the mutation.

Figure 2. Change in relative fitness in the mutation accumulation (MA) lines from *C. elegans* and *C. briggsae* in the Baer et al. (2005) MA assay. The figure was modified from that of Baer et al. (2005). The N2 strain of *C. elegans* is identified by the gray triangles and white connecting arrows; the HK104 strain of *C. briggsae* is indicated by the black squares and gray arrows. Error bars denote standard error for estimates of relative fitness. The mean fitness of each unmutated (generation 0) ancestor was set to 0, negative numbers on the y axis indicate decreases in fitness in the MA lines in comparison to the unmutated ancestral control.

Figure 3. Experiment 1, proportion of N2 MA and AC nematodes alive at each time point on heat stress and control. Data are the proportion of nematodes alive (out of 10 nematodes) at each time point and are graphed as mean ± standard error. Ancestral control (AC) lines are the black bars, mutation accumulation (MA) lines are the gray bars. 'H' indicates the number of hours in control conditions (20°C) or heat stress (35°C). Different letters above the bars indicate statistically significant differences.

Figure 4. Experiment 1, proportion of HK104 MA and AC nematodes alive at each time point on heat stress and control. Data are the proportion of nematodes alive (out of 10 nematodes) at each time point and are graphed as mean ± standard error and averaged across mutation accumulation (MA) lines or ancestral control (AC) pseudolines. Ancestral control lines are the black bars, mutation accumulation (MA) lines are the gray bars. 'H' indicates the number of hours in control conditions (20°C) or heat stress (35°C).

Figure 5. Experiment 1, proportion of N2 MA and AC nematodes surviving to a given day (control not included) on paraquat stress. Data are the proportion of nematodes alive (out of 10 nematodes) at each time point and are graphed as mean ± standard error and averaged across mutation accumulation (MA) lines or ancestral control (AC) pseudolines. Ancestral control lines are the black bars, mutation accumulation (MA) lines are the gray bars.

Figure 6. Experiment 1, proportion of N2 nematodes surviving over the entire experiment on NGM and paraquat stress. The data indicate the total proportion of hatched nematodes in each mutation accumulation (MA) line or ancestral control (AC) pseudoline that survived until they developed to adulthood during the experiment. Data are graphed as mean ± standard error. Results from control conditions (nematode growth medium, NGM) are graphed as gray bars and results from paraquat-containing plates are presented as hatched bars.

Figure 7. Experiment 1, development score of N2 lines at day 3 on NGM and paraquat. The data indicate the mean development score of hatched nematodes in each mutation accumulation (MA) line or ancestral control (AC) pseudoline on day 3. Data are graphed as mean ± standard error. Results from control conditions (nematode growth medium,

NGM) are graphed as gray bars and results from paraquat-containing plates are presented as hatched bars.

Figure 8. Experiment 1, proportion of HK104 MA and AC nematodes surviving to a given day (control not included) on paraquat. Data are the proportion of nematodes alive (out of 10 nematodes) at each time point and are graphed as mean ± standard error and averaged across mutation accumulation (MA) lines or ancestral control (AC) pseudolines. Ancestral control lines are the black bars, mutation accumulation (MA) lines are the gray bars.

Figure 9. Experiment 1, proportion of HK104 lines surviving over the entire experiment on NGM and paraquat. The data indicate the total proportion of hatched nematodes in each mutation accumulation (MA) line or ancestral control (AC) pseudoline that survived until they developed to adulthood during the experiment. Data are graphed as mean ± standard error. Results from control conditions (nematode growth medium, NGM) are graphed as gray bars and results from paraquat-containing plates are presented as hatched bars.

Figure 10. Experiment 1, development score of HK104 lines at day 3 on NGM and paraquat. The data indicate the average development score in each mutation accumulation (MA) line or ancestral control (AC) pseudoline on day 3. Data are graphed as mean ± standard error. Results from control conditions (nematode growth medium, NGM) are graphed as gray bars and results from paraquat-containing plates as hatched bars.

Figure 11. Experiment 1, proportion of N2 MA and AC nematodes surviving to a given day (control not included) on salt. Data are the proportion of nematodes alive (out of 5 nematodes) at each time point and are graphed as mean ± standard error and averaged

across mutation accumulation (MA) lines (black bars) or ancestral control (AC) pseudolines (gray bars).

Figure 12. Experiment 1, proportion of N2 lines surviving over the entire experiment on NGM and salt. The data indicate the proportion of nematodes from each mutation accumulation (MA) line or ancestral control (AC) pseudoline that survived to adulthood during the assay. Data are graphed as mean ± standard error. Results from control conditions (nematode growth medium, NGM) are graphed as gray bars and results from high salt plates as hatched bars.

Figure 13. Experiment 1, development score of N2 lines at day 3 on NGM and salt. The data indicate the development score of each mutation accumulation (MA) line or ancestral control (AC) pseudoline on day 3. Data are graphed as mean ± standard error. Results from control conditions (nematode growth medium, NGM) are graphed as gray bars and results from high salt plates as hatched bars.

Figure 14. Experiment 1, proportion of HK104 MA and AC nematodes surviving to a given day (control not included) on salt. Data are the proportion of nematodes alive (out of 5 nematodes) at each time point and are graphed as mean ± standard error and averaged across mutation accumulation (MA) lines (black bars) or ancestral control (AC) pseudolines (gray bars).

Figure 15. Experiment 1, proportion of HK104 lines surviving over the entire experiment on NGM and salt. The data indicate the total proportion of hatched nematodes from each accumulation (MA) line or ancestral control (AC) pseudoline that survived and developed to adulthood during the assay. Data are graphed as mean ± standard error.

Results from control conditions (nematode growth medium, NGM) are graphed as gray bars and results from high salt plates as hatched bars.

Figure 16. Experiment 1, development score of HK104 lines at day 3 on NGM and salt. The data indicate development score from each accumulation (MA) line or ancestral control (AC) pseudoline on day 3. Data are graphed as mean ± standard error. Results from control conditions (nematode growth medium, NGM) are graphed as gray bars and results from high salt plates as hatched bars.

Figure 17. Experiment 2, proportion of nematodes alive to a given time (control included) on heat stress. The data are the proportion of nematodes alive (out of 10 nematodes) at each time point and are graphed as mean ± standard error. Ancestral control (AC) lines are the dark gray bars, high fitness (High) mutation accumulation (MA) lines are the light gray bars and low fitness (Low) MA lines are the white bars. 'H' indicates the number of hours in control conditions (20°C) or heat stress (35°C).

Figure 18. Experiment 2, proportion of nematodes alive to 18 hours on heat control. The data indicate the proportion of nematodes (out of 10 nematodes) alive at 18 hours on heat control. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control (AC) are graphed as filled circles, high fitness (High) mutation accumulation (MA) lines are graphed as open triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

Figure 19. Experiment 2, proportion of nematodes alive to 18 hours on heat stress. The data indicate the proportion of nematodes (out of 10 nematodes) alive at 18 hours on heat stress. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control (AC) are graphed as filled circles, high fitness (High) mutation

accumulation (MA) lines are graphed as open triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

Figure 20. Experiment 2, proportion of nematodes surviving to day 3 on NGM, paraquat assay. The data indicate the proportion of nematodes (out of 10 nematodes) that survived to day 3. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control (AC) are graphed as filled circles, high fitness (High) mutation accumulation (MA) lines are graphed as open triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

Figure 21. Experiment 2, proportion of nematodes surviving to a given day (control not included) on paraquat. The data are the proportion of nematodes alive (out of 10 nematodes) at each time point and are graphed as mean ± standard error. Ancestral control (AC) lines are the dark gray bars, high fitness (High) mutation accumulation (MA) lines are the light gray bars and low fitness (Low) MA lines are the white bars.

Figure 22. Experiment 2, proportion of nematodes surviving to day 3 on paraquat. The data indicate the proportion of nematodes (out of 10 nematodes) that survived to day 3. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control (AC) are graphed as filled circles, high fitness (High) mutation accumulation (MA) lines are graphed as open triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

Figure 23. Experiment 2, the number of days needed to develop to adulthood on NGM, paraquat assay. The data indicate number of days needed for nematodes to develop to adulthood. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control (AC) are graphed as filled circles, high fitness (High) mutation

accumulation (MA) lines are graphed as open triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

Figure 24. Experiment 2, proportion of hatched nematodes that were adults on day 3 on NGM, paraquat assay. The data indicate the proportion of hatched nematodes that were adults on day 3. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control (AC) are graphed as filled circles, high fitness (High) mutation accumulation (MA) lines are graphed as open triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

Figure 25. Experiment 2, the number of days needed to develop to adulthood on paraquat. The data indicate number of days needed for nematodes to develop to adulthood. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control (AC) are graphed as filled circles, high fitness (High) mutation accumulation (MA) lines are graphed as open triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

Figure 26. Experiment 2, proportion of hatched nematodes that were adults on day 3 on paraquat. The data indicate the proportion of hatched nematodes that were adults on day 3. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control (AC) are graphed as filled circles, high fitness (High) mutation accumulation (MA) lines are graphed as open triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

Figure 27. Experiment 2, proportion of nematodes surviving to adulthood during the entire experiment on NGM and paraquat. The data indicate the total proportion of hatched nematodes that survived and developed to adulthood on control conditions

(nematode growth medium, NGM, gray bars) and on paraquat-containing plates (hatched bars). Data are presented as mean ± standard error within fitness category: AC, ancestral control; High, high fitness mutation accumulation (MA) lines; Low, low fitness MA lines.

Figure 28. Experiment 2, proportion of nematodes surviving to adulthood during the entire experiment on NGM, paraquat assay. The data indicate the proportion of hatched nematodes that survived to develop to adulthood. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control (AC) are graphed as filled circles, high fitness (High) mutation accumulation (MA) lines are graphed as open triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

Figure 29. Experiment 2, proportion of nematodes surviving to adulthood during the entire experiment on paraquat. The data indicate the proportion of hatched nematodes that survived to develop to adulthood. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control (AC) are graphed as filled circles, high fitness (High) mutation accumulation (MA) lines are graphed as open triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

Figure 30. Experiment 2, development score on day 3 on NGM, paraquat assay. The data indicate the development score of nematodes on day 3. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control (AC) are graphed as filled circles, high fitness (High) mutation accumulation (MA) lines are graphed as open

triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

Figure 31. Experiment 2, development score over the entire experiment on paraquat. The data are the development scores averaged across mutation accumulation (MA) lines and ancestral control (AC) pseudolines at each time point and are graphed as mean ± standard error. Ancestral control lines are the dark gray bars, high fitness (High) MA lines are the light gray bars and low fitness (Low) MA lines are the white bars.

Figure 32. Experiment 2, development score on day 3 on paraquat. The data indicate the development score averaged across mutation accumulation (MA) lines and ancestral control (AC) pseudolines. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control are graphed as filled circles, high fitness (High) MA lines are graphed as open triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

Figure 33. Experiment 2, proportion of nematodes surviving to a given day (control not included) on salt. The data are the proportion of surviving nematodes (out of 5 nematodes) averaged across mutation accumulation (MA) lines and ancestral control (AC) pseudolines at each time point and are graphed as mean ± standard error. Ancestral control lines are the dark gray bars, high fitness (High) MA lines are the light gray bars and low fitness (Low) MA lines are the white bars.

Figure 34. Experiment 2, proportion of nematodes surviving to day 3 on NGM, salt assay. The data indicate the proportion of nematodes, averaged across mutation accumulation (MA) lines and ancestral control (AC) pseudolines, that survived to day 3. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral

control are graphed as filled circles, high fitness (High) MA lines are graphed as open triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

Figure 35. Experiment 2, proportion of nematodes surviving to day 3 on salt. The data indicate the proportion of nematodes, averaged across mutation accumulation (MA) lines and ancestral control (AC) pseudolines, that survived to day 3. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control are graphed as filled circles, high fitness (High) MA lines are graphed as open triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

Figure 36. Experiment 2, proportion of hatched nematodes that were adults on day 3 on NGM, salt assay. The data indicate the proportion of hatched nematodes, averaged across mutation accumulation (MA) lines and ancestral control (AC) pseudolines, that developed to adulthood on day 3. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control are graphed as filled circles, high fitness (High) MA lines are graphed as open triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

Figure 37. Experiment 2, the number of days needed to develop to adulthood on salt. The data indicate the number of days needed to develop to adulthood, averaged across mutation accumulation (MA) lines and ancestral control (AC) pseudolines. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control are graphed as filled circles, high fitness (High) MA lines as open triangles and low fitness (Low) MA lines as filled circles. Data are presented as mean ± standard error.

Figure 38. Experiment 2, the proportion of hatched nematodes that were adults on day 5 on salt. The data indicate the proportion of hatched nematodes, averaged across mutation accumulation (MA) lines and ancestral control (AC) pseudolines, that developed to adulthood on day 5. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control are graphed as filled circles, high fitness (High) MA lines as open triangles and low fitness (Low) MA lines as filled circles. Data are presented as mean ± standard error.

Figure 39. Experiment 2, proportion of nematodes surviving to adulthood during the entire experiment on NGM, salt assay. The data indicate the proportion of nematodes, averaged across mutation accumulation (MA) lines and ancestral control (AC) pseudolines, that survived and developed to adulthood. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control are graphed as filled circles, high fitness (High) MA lines as open triangles and low fitness (Low) MA lines as filled circles. Data are presented as mean ± standard error.

Figure 40. Experiment 2, proportion of nematodes surviving over the entire experiment on NGM and salt. The data indicate the total proportion of nematodes that survived and developed to adulthood on control conditions (nematode growth medium, NGM, gray bars) and on high salt plates (hatched bars). Data are presented as mean ± standard error within fitness category: AC, ancestral control; High, high fitness mutation accumulation (MA) lines; Low, low fitness MA lines.

Figure 41. Experiment 2, proportion of nematodes surviving to adulthood during the entire experiment on salt. The data indicate the proportion of nematodes, averaged across mutation accumulation (MA) lines and ancestral control (AC) pseudolines, that survived

and developed to adulthood. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control are graphed as filled circles, high fitness (High) MA lines as open triangles and low fitness (Low) MA lines as filled circles. Data are presented as mean ± standard error.

Figure 42. Experiment 2, development score on day 3 on NGM, salt assay. The data indicate development score at day 3, averaged across mutation accumulation (MA) lines and ancestral control (AC) pseudolines. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control are graphed as filled circles, high fitness (High) MA lines as open triangles and low fitness (Low) MA lines as filled circles. Data are presented as mean ± standard error.

Figure 43. Experiment 2, development score over the entire experiment on salt. The data are development score averaged across mutation accumulation (MA) lines and ancestral control (AC) pseudolines at each time point and are graphed as mean ± standard error. Ancestral control lines are the dark gray bars, high fitness (High) MA lines are the light gray bars and low fitness (Low) MA lines are the white bars.

Figure 44. Experiment 2, development score on day 3 on salt. The data indicate development score at day 3, averaged across mutation accumulation (MA) lines and ancestral control (AC) pseudolines. Data are graphed versus block to facilitate comparisons between the blocks. Ancestral control are graphed as filled circles, high fitness (High) MA lines are graphed as open triangles and low fitness (Low) MA lines are graphed as filled circles. Data are presented as mean ± standard error.

FIGURES

Figure 1. Mutation accumulation flow chart in *C. elegans*.

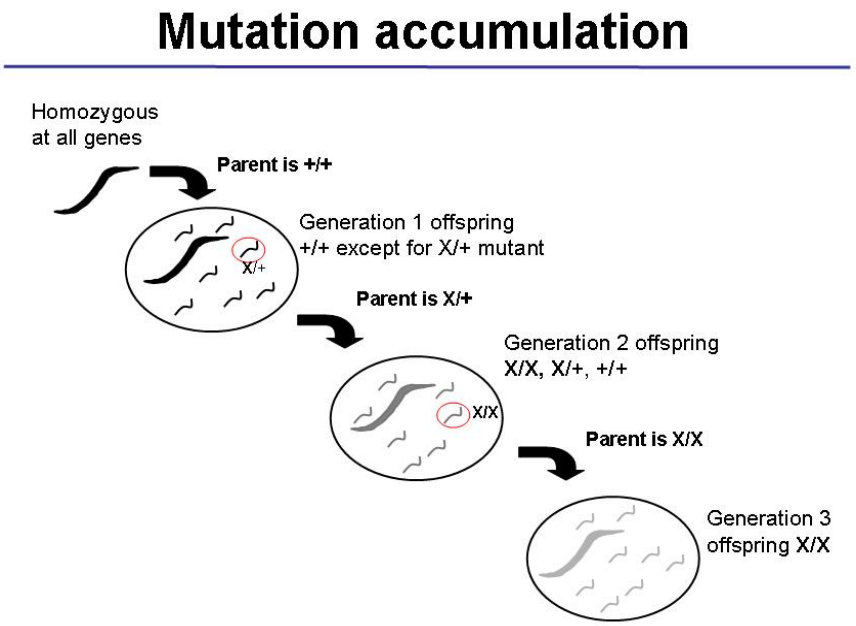


Figure 2. Average fitness decreases in *C. elegans* and *C. briggsae* MA lines compared to the fitness of unmutated ancestor.

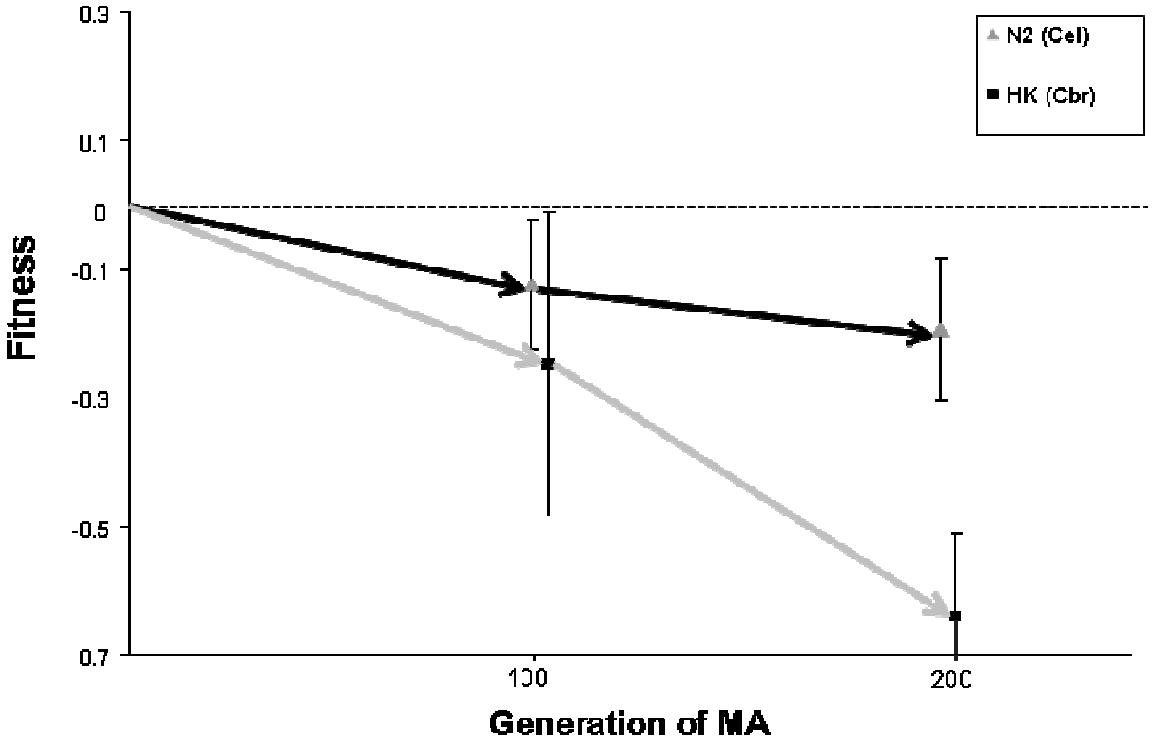


Figure 3. Experiment 1, proportion of N2 MA and AC nematodes alive at each time point on heat stress and control.

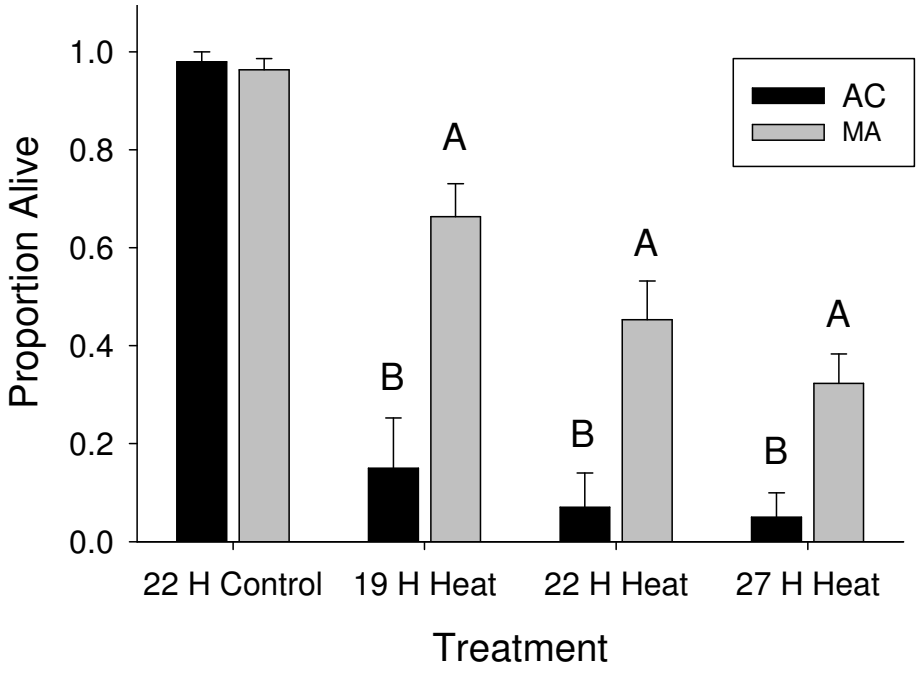


Figure 4. Experiment 1, proportion of HK104 MA and AC alive at each time point on heat stress and control.

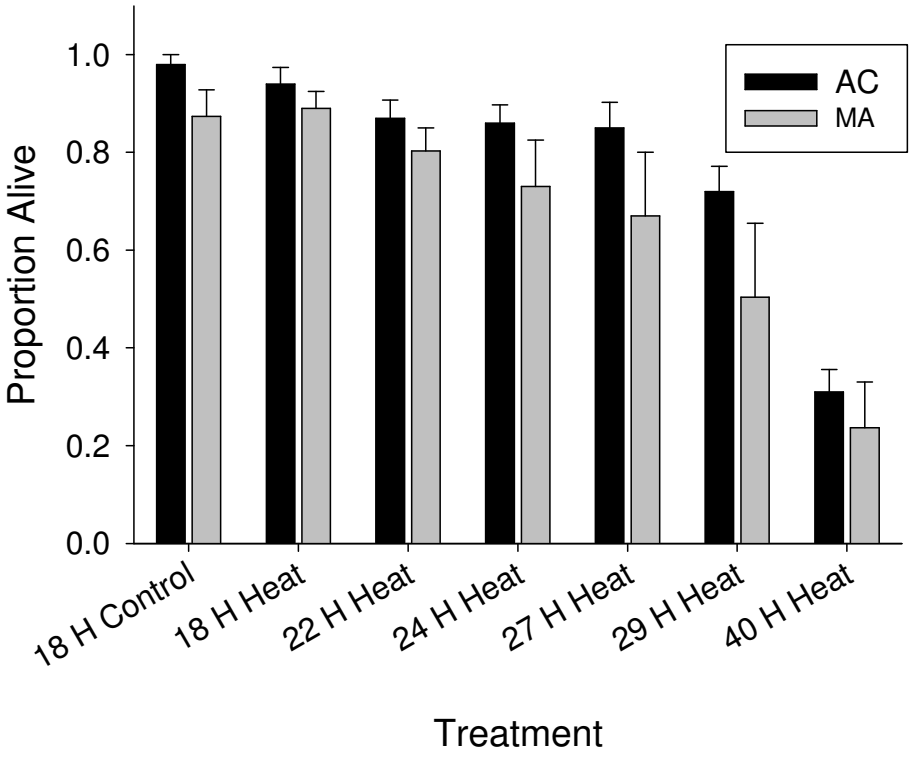


Figure 5. Experiment 1, proportion of N2 MA and AC nematodes surviving to a given day (control not included) on paraquat stress.

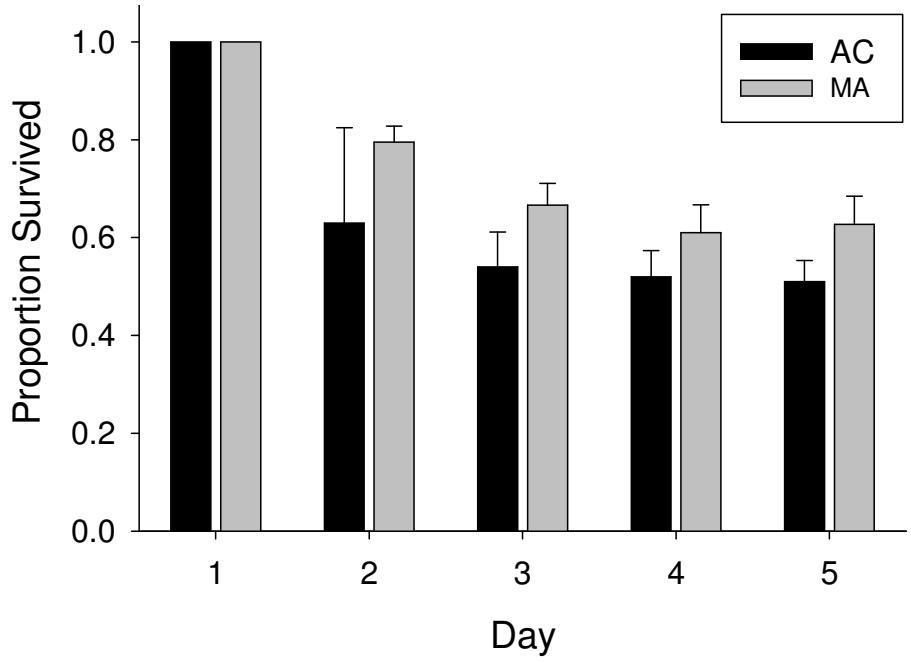


Figure 6. Experiment 1, proportion of N2 sequenced lines surviving over the entire experiment on NGM and paraquat stress.

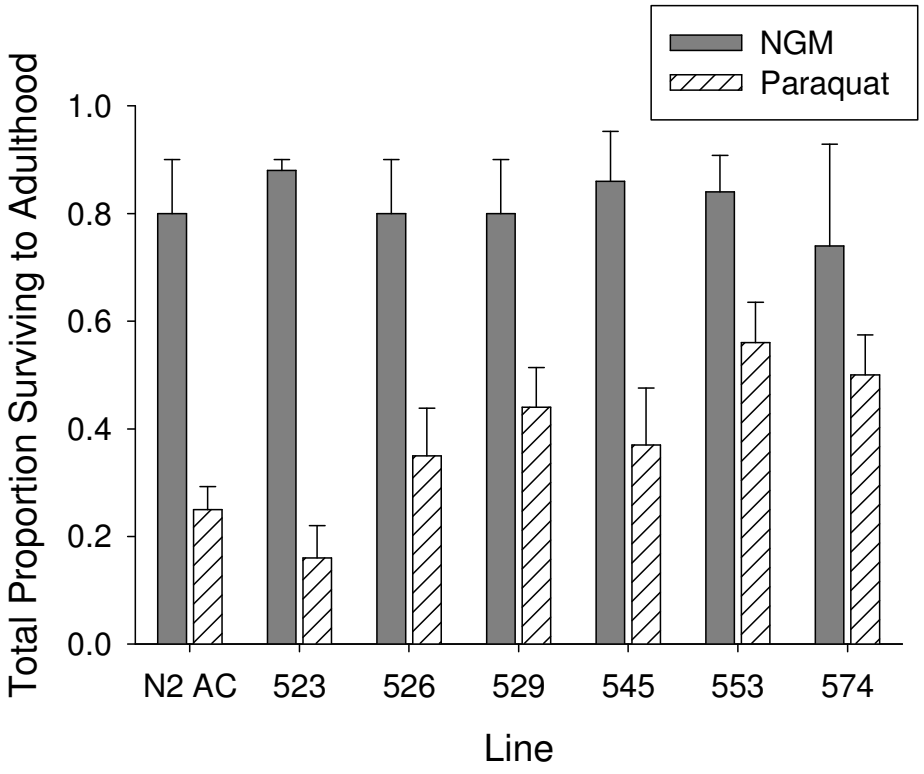


Figure 7. Experiment 1, development score of N2 lines at day 3 on NGM and paraquat.

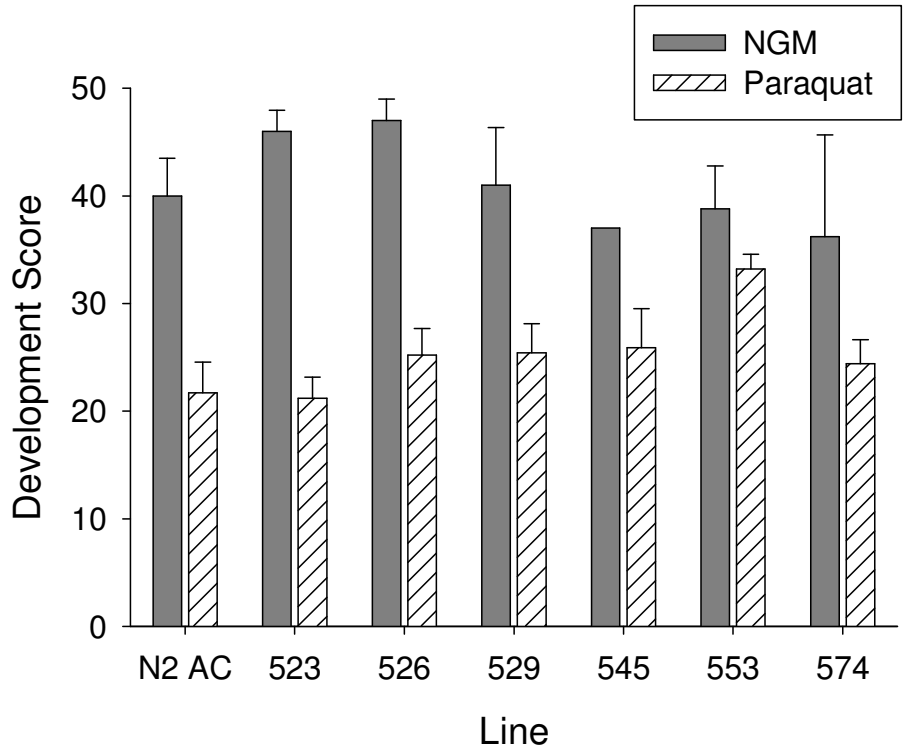


Figure 8. Experiment 1, proportion of HK104 MA and AC nematodes surviving to a given day (control not included) on paraquat.

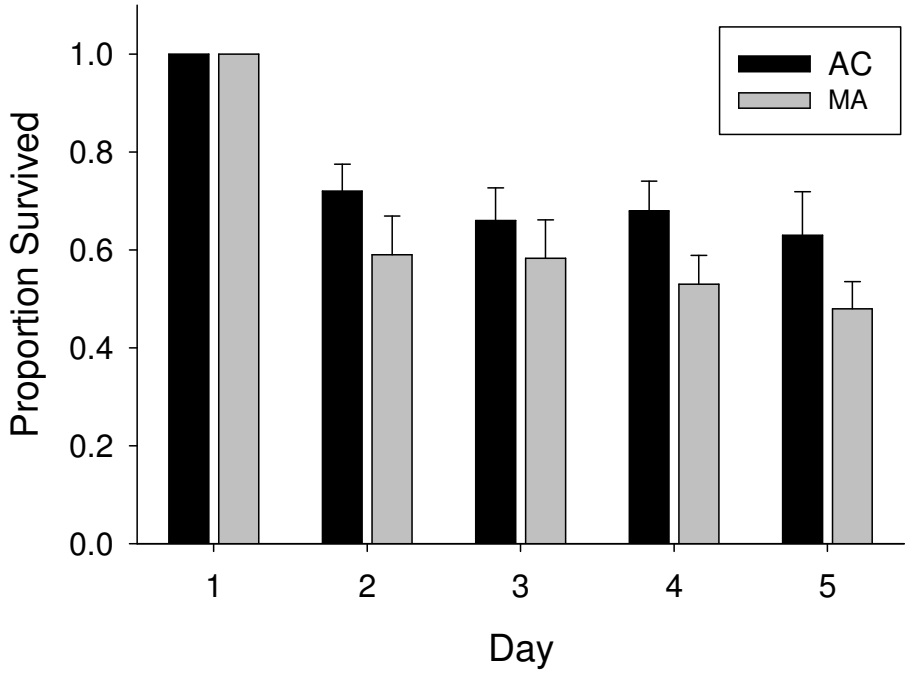


Figure 9. Experiment 1, proportion of HK104 lines surviving over the entire experiment on NGM and paraquat.

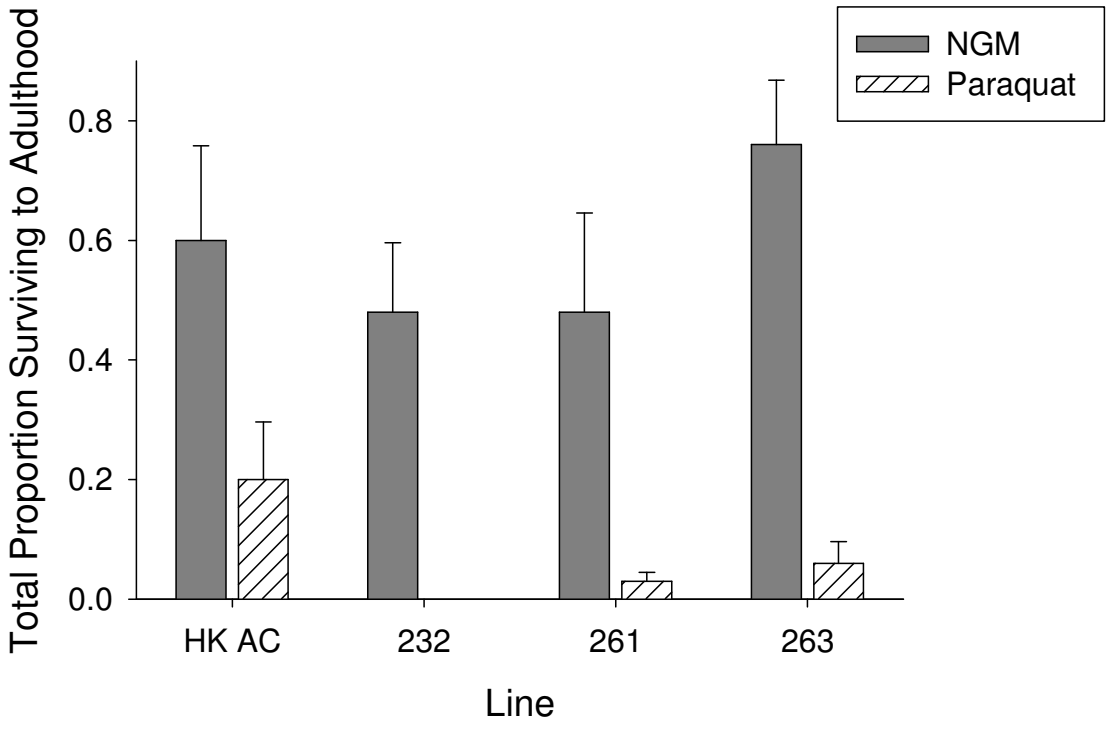


Figure 10. Experiment 1, development score of HK104 lines at day 3 on NGM and paraquat.

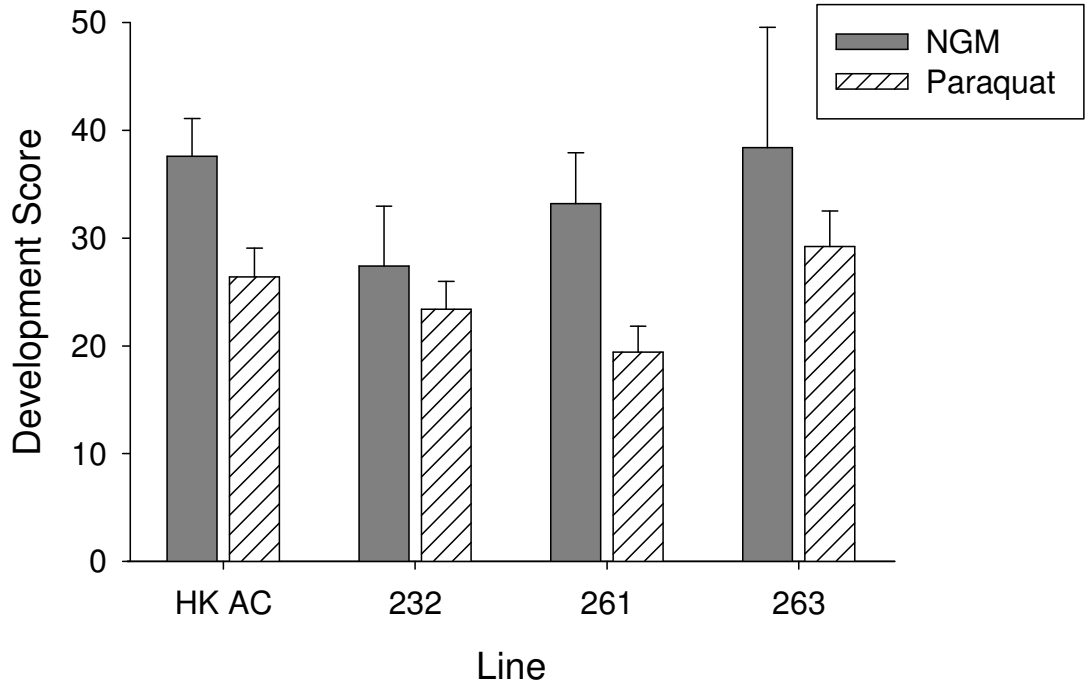


Figure 11. Experiment 1, proportion of N2 MA and AC nematodes surviving to a given day (control not included) on salt.

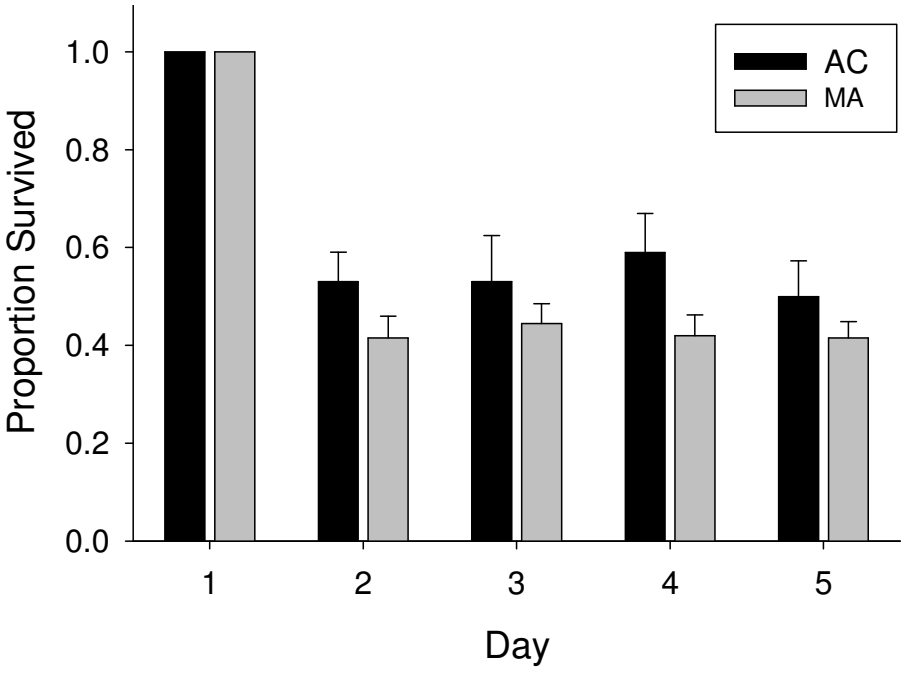


Figure 12. Experiment 1, proportion of N2 lines surviving over the entire experiment on NGM and salt.

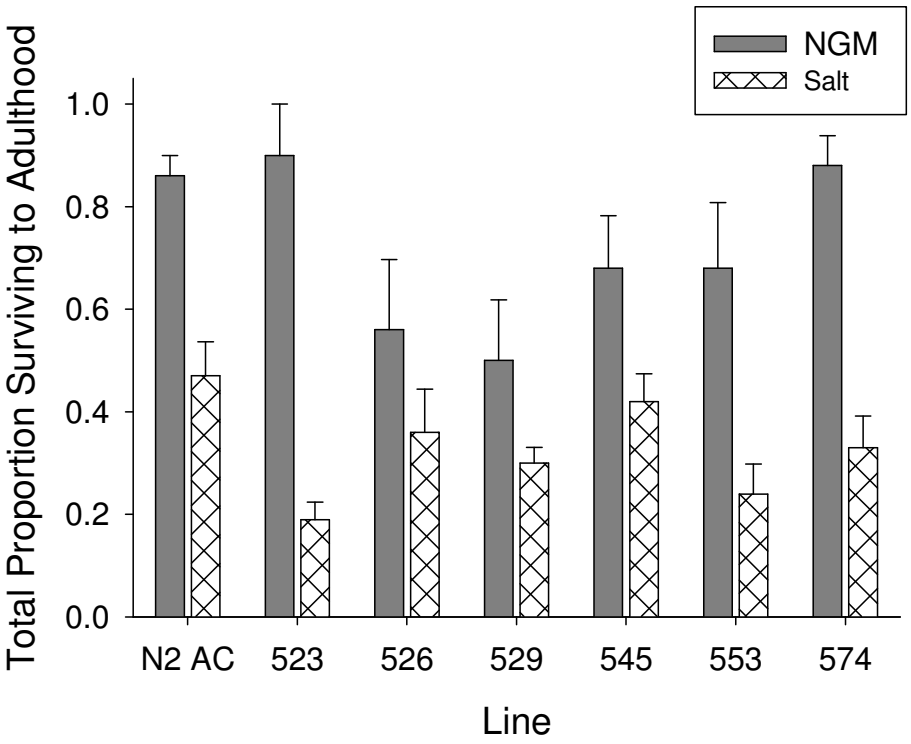


Figure 13. Experiment 1, development score of N2 lines at day 3 on NGM and salt.

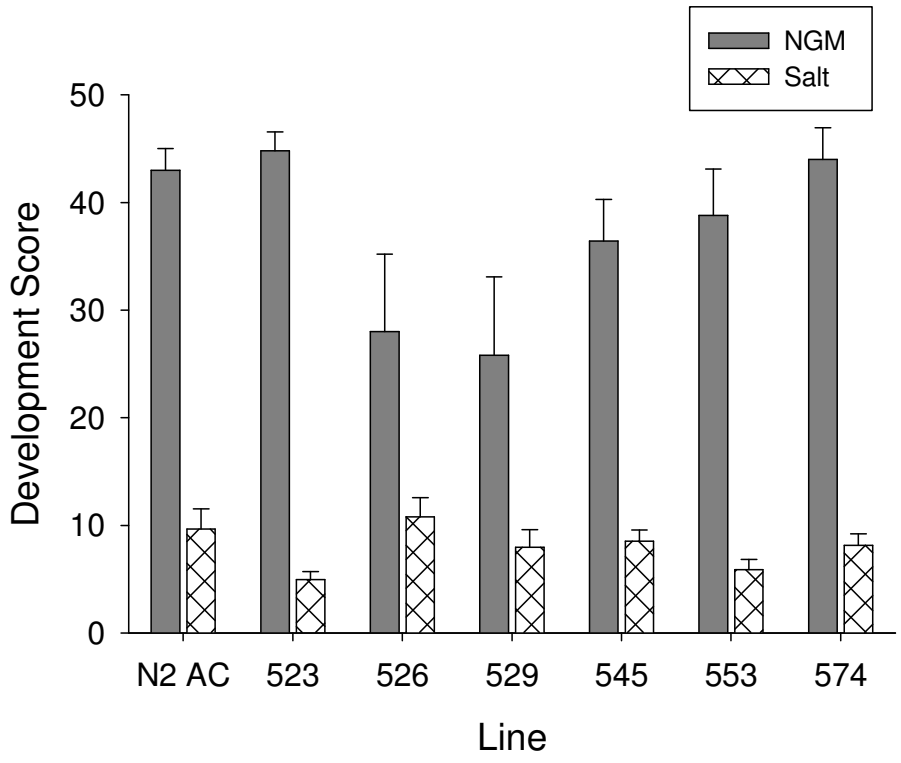


Figure 14. Experiment 1, proportion of HK104 MA and AC nematodes surviving to a given day (control not included) on salt.

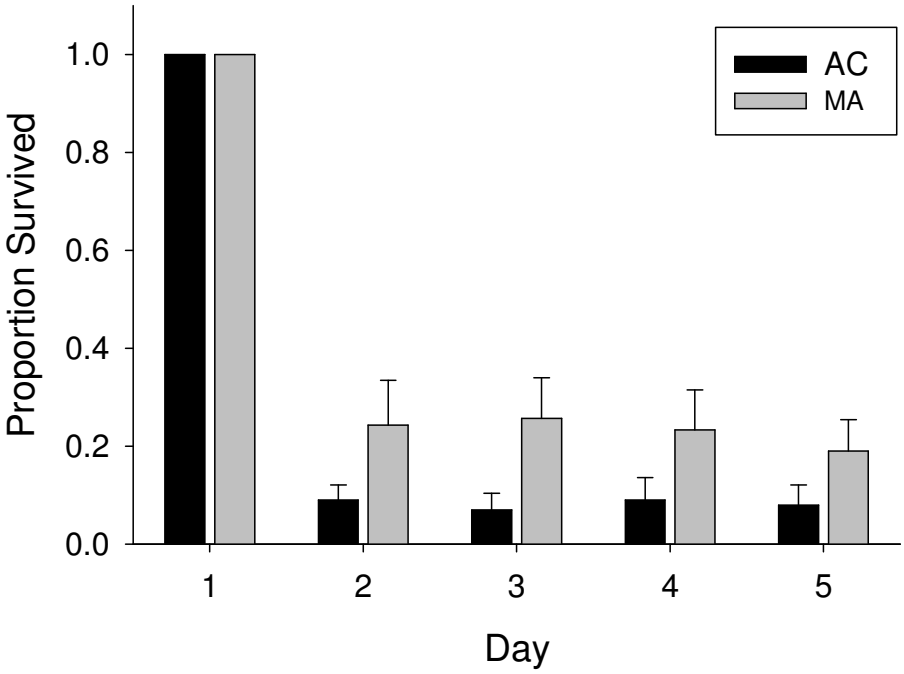


Figure 15. Experiment 1, proportion of HK104 lines surviving over the entire experiment on NGM and salt.

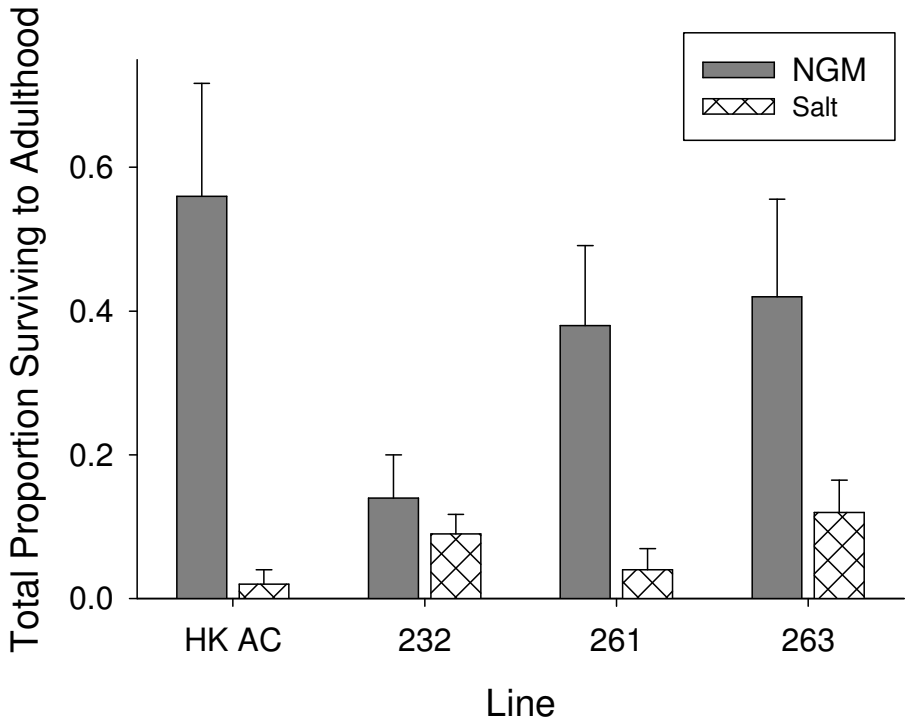


Figure 16. Experiment 1, development score of HK104 lines at day 3 on NGM and salt.

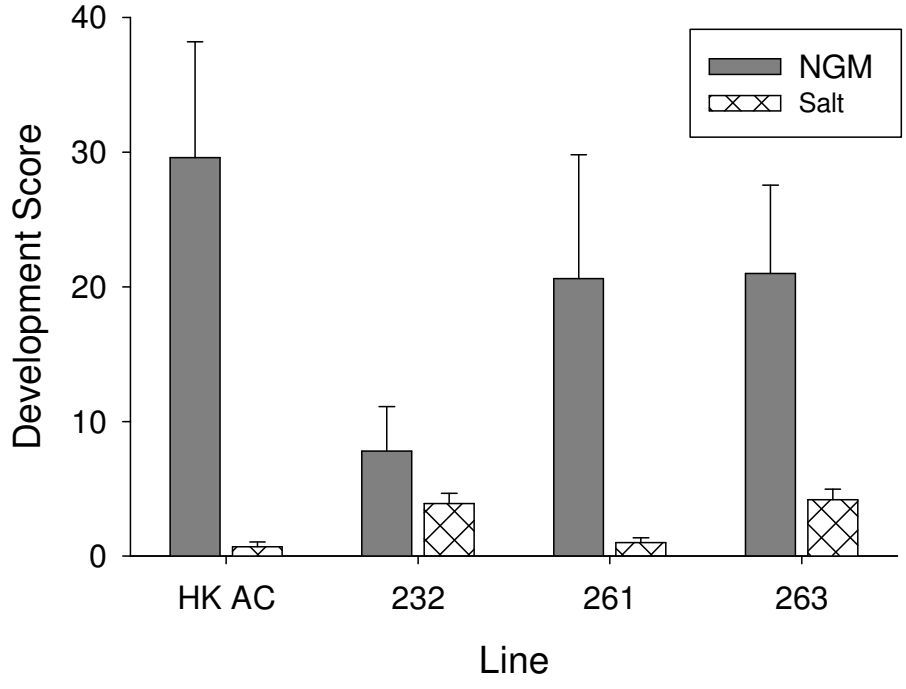


Figure 17. Experiment 2, proportion of nematodes alive to a given time (control included) on heat stress.

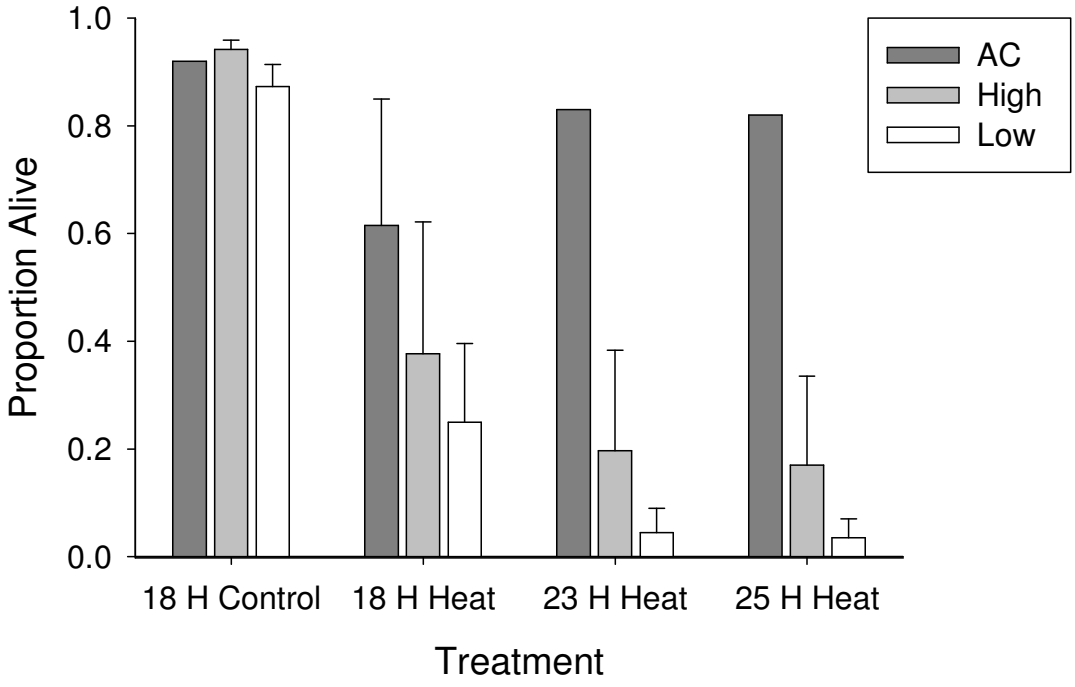


Figure 18. Experiment 2, proportion of nematodes alive to 18 hours on heat control.

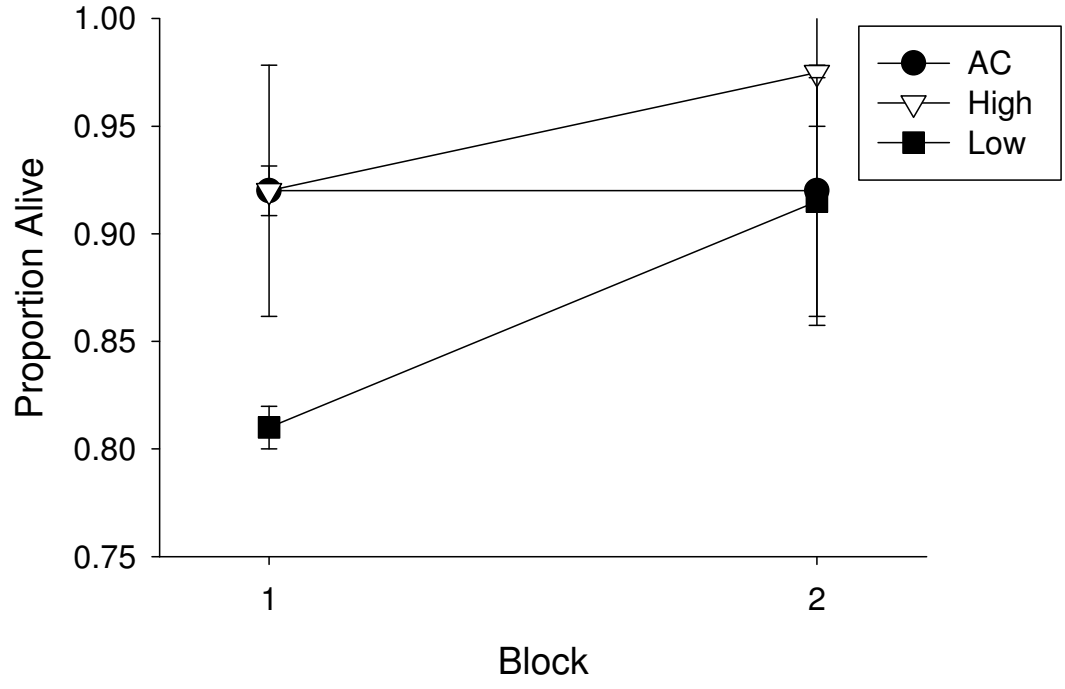


Figure 19. Experiment 2, proportion of nematodes alive to 18 hours on heat stress.

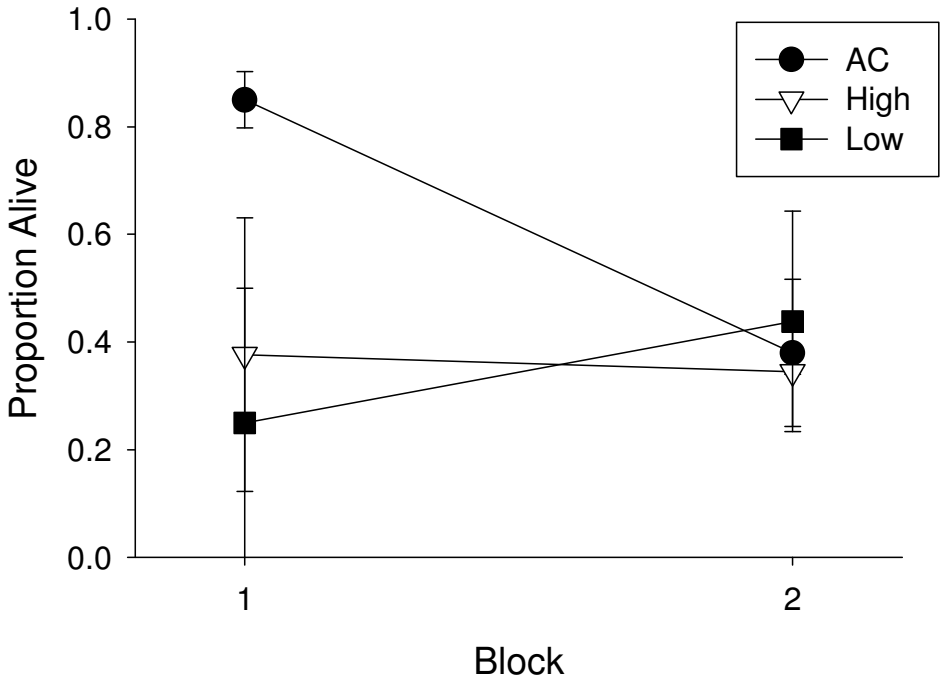


Figure 20. Experiment 2, proportion of nematodes surviving to day 3 on NGM, paraquat assay.

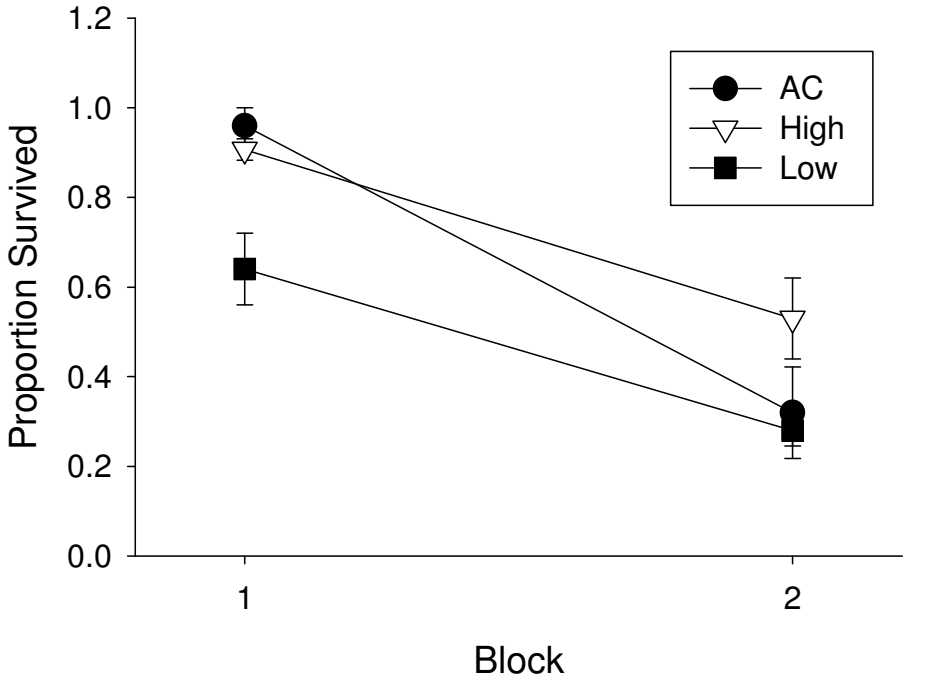


Figure 21. Experiment 2, proportion of nematodes surviving to a given day (control not included) on paraquat.

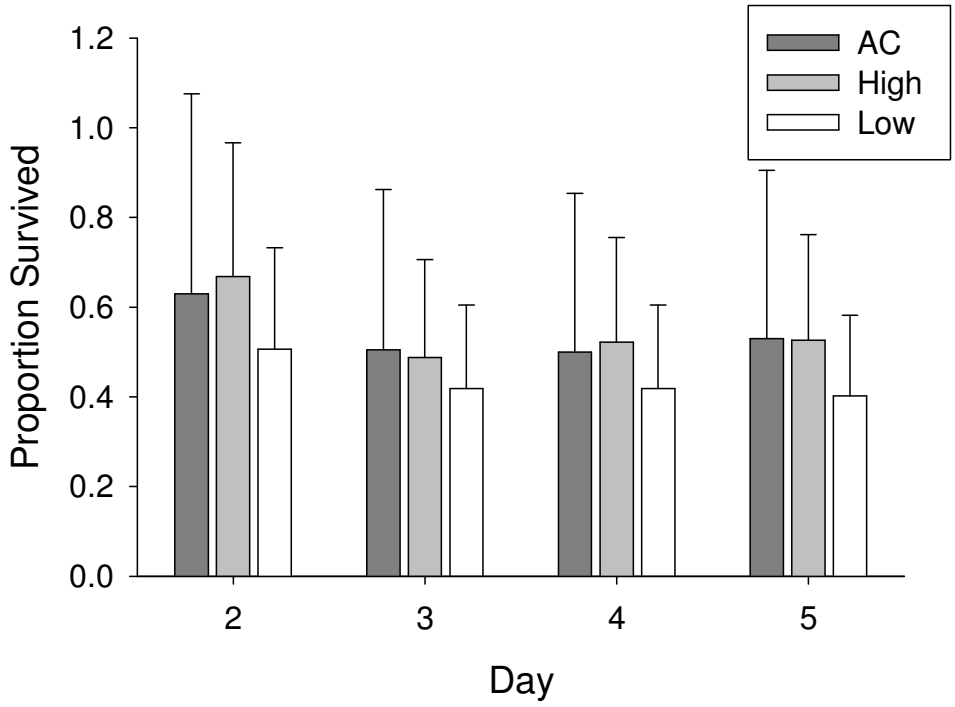


Figure 22. Experiment 2, proportion of nematodes surviving to day 3 on paraquat.

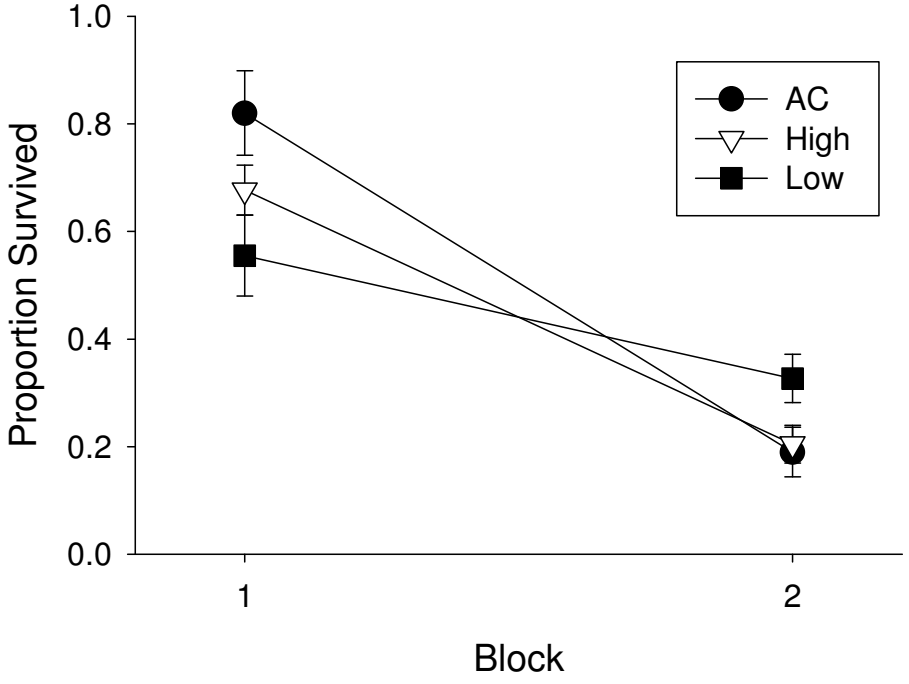


Figure 23. Experiment 2, the number of days needed to develop to adulthood on NGM, paraquat assay.

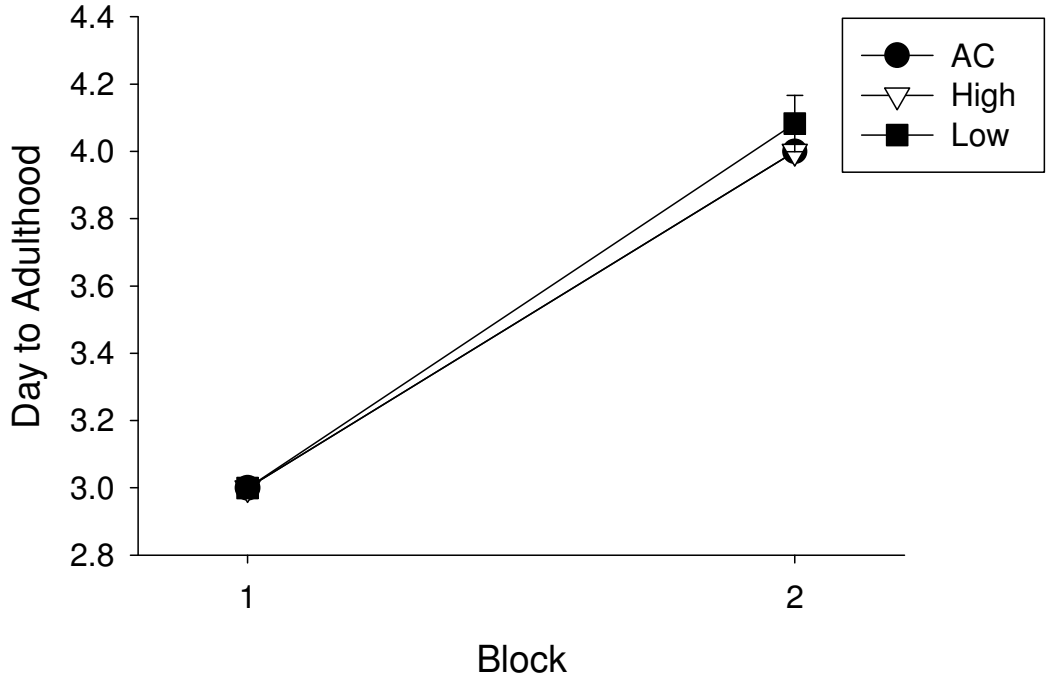


Figure 24. Experiment 2, proportion of hatched nematodes that were adults on day 3 on NGM, paraquat assay.

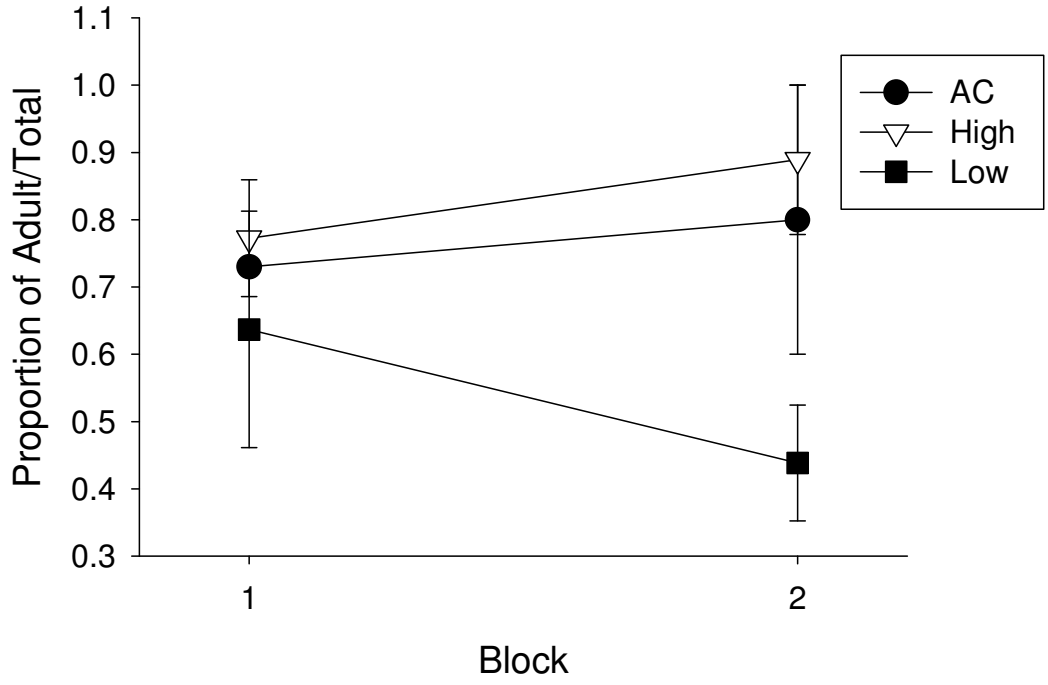


Figure 25. Experiment 2, the number of days needed to develop to adulthood on paraquat.

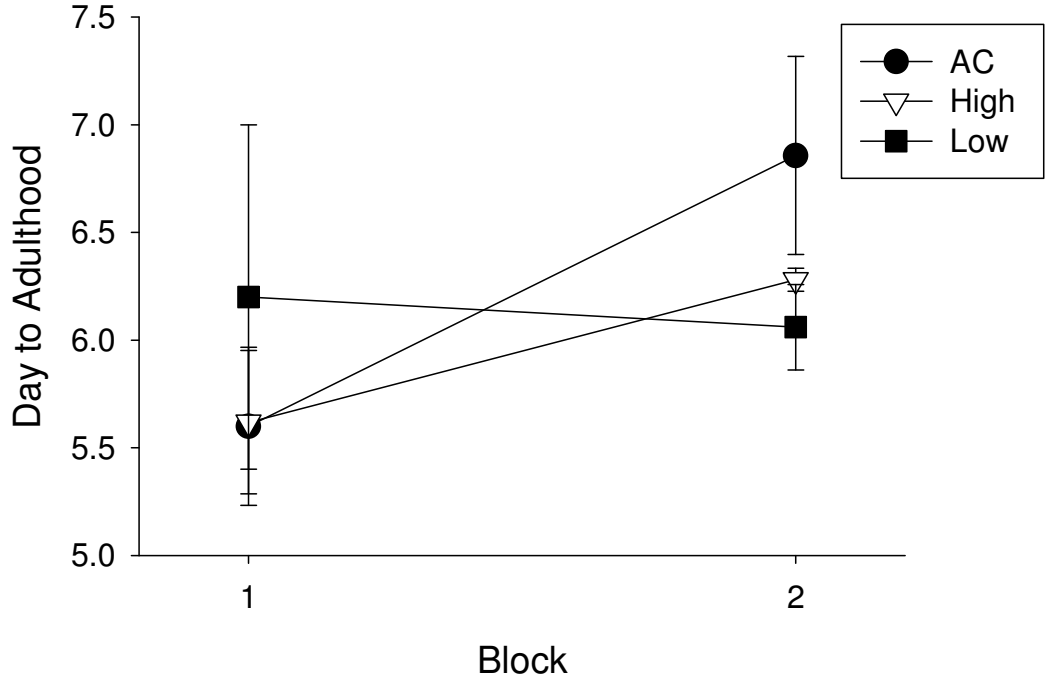


Figure 26. Experiment 2, proportion of hatched nematodes that were adults on day 3 on paraquat.

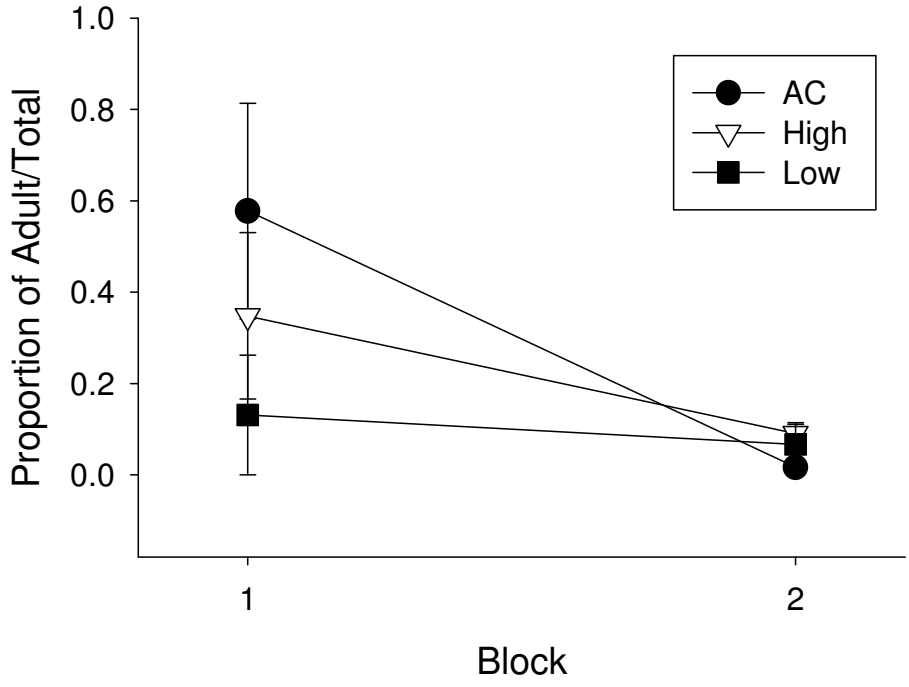


Figure 27. Experiment 2, proportion of nematodes surviving to adulthood during the entire experiment on NGM and paraquat.

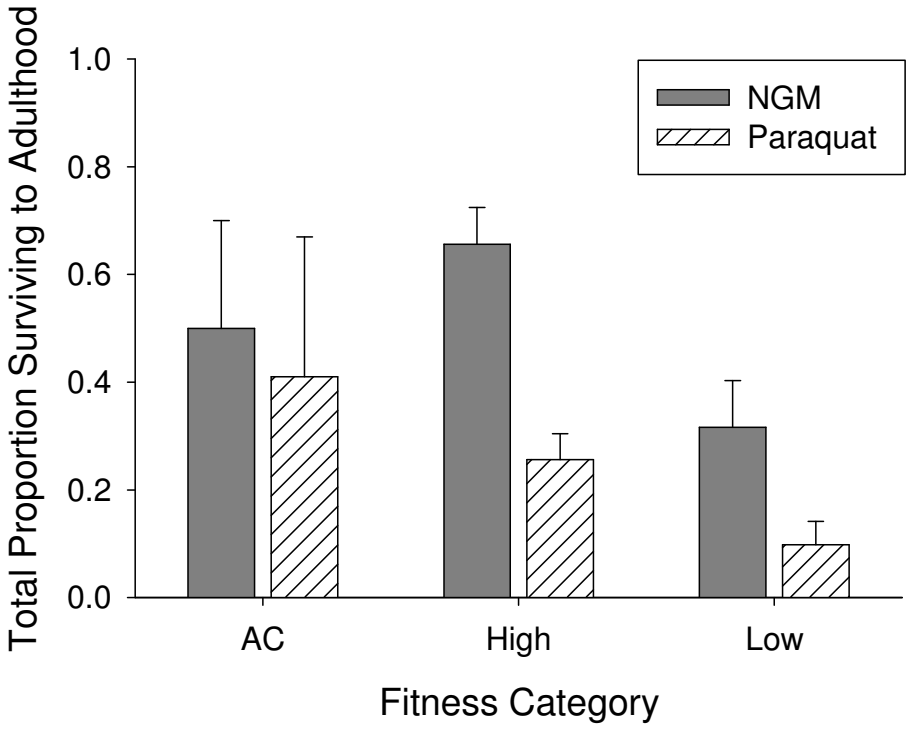


Figure 28. Experiment 2, proportion of nematodes surviving to adulthood during the entire experiment on NGM, paraquat assay.

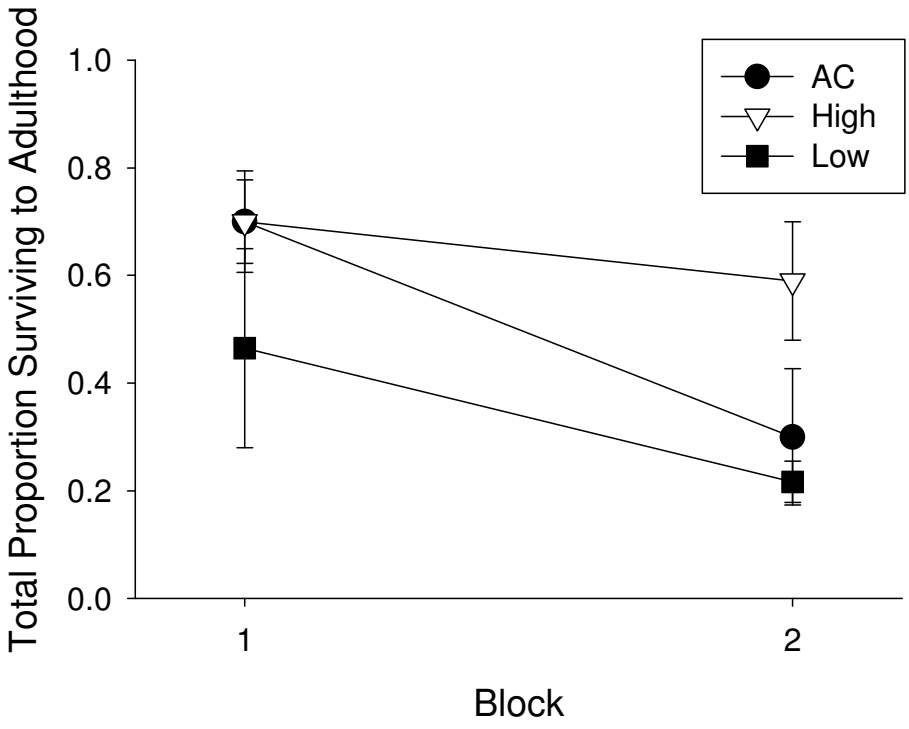


Figure 29. Experiment 2, proportion of nematodes surviving to adulthood during the entire experiment on paraquat.

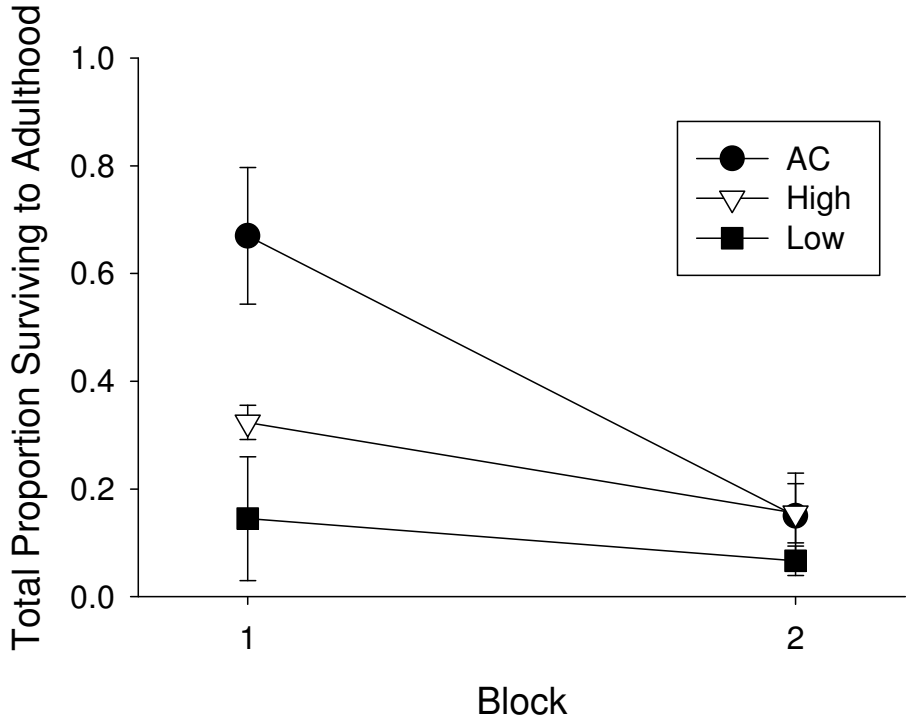


Figure 30. Experiment 2, development score on day 3 on NGM, paraquat assay.

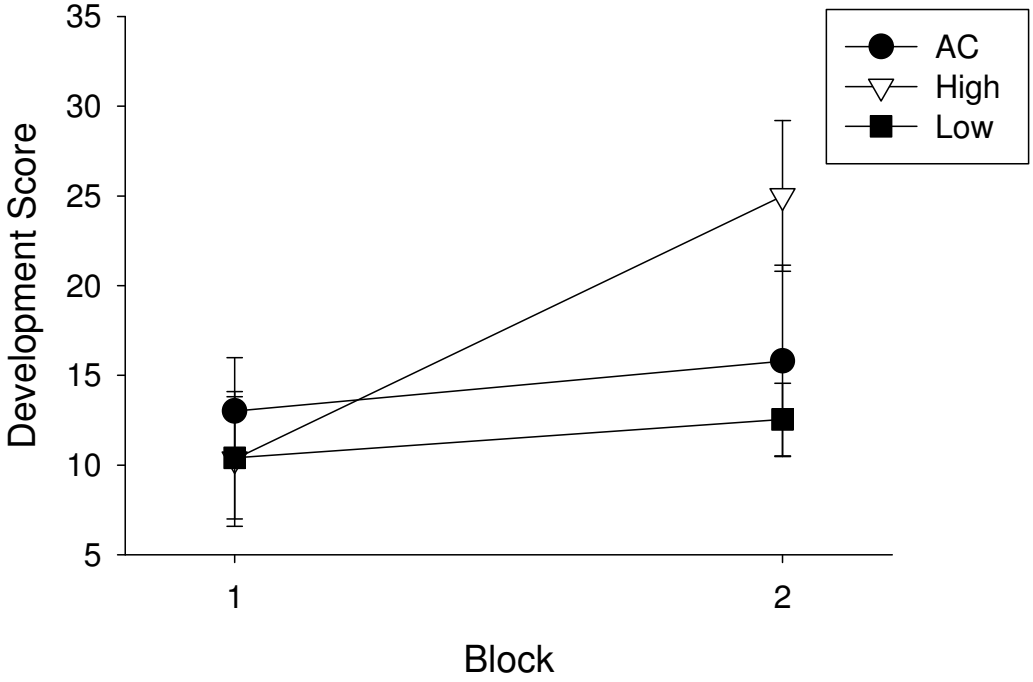


Figure 31. Experiment 2, development score over the entire experiment on paraquat.

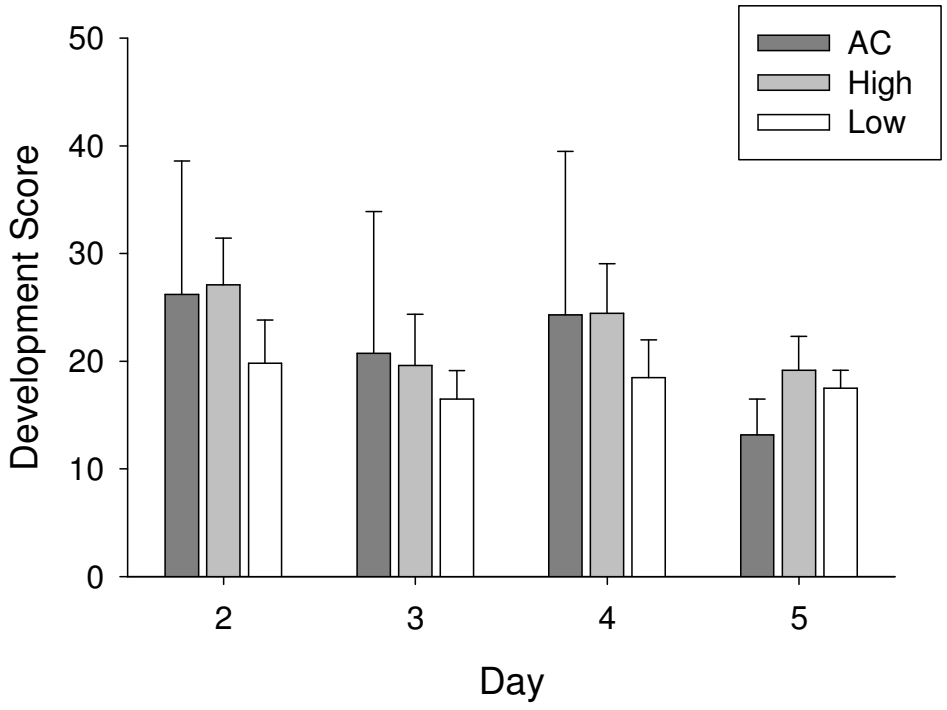


Figure 32. Experiment 2, development score on day 3 on paraquat.

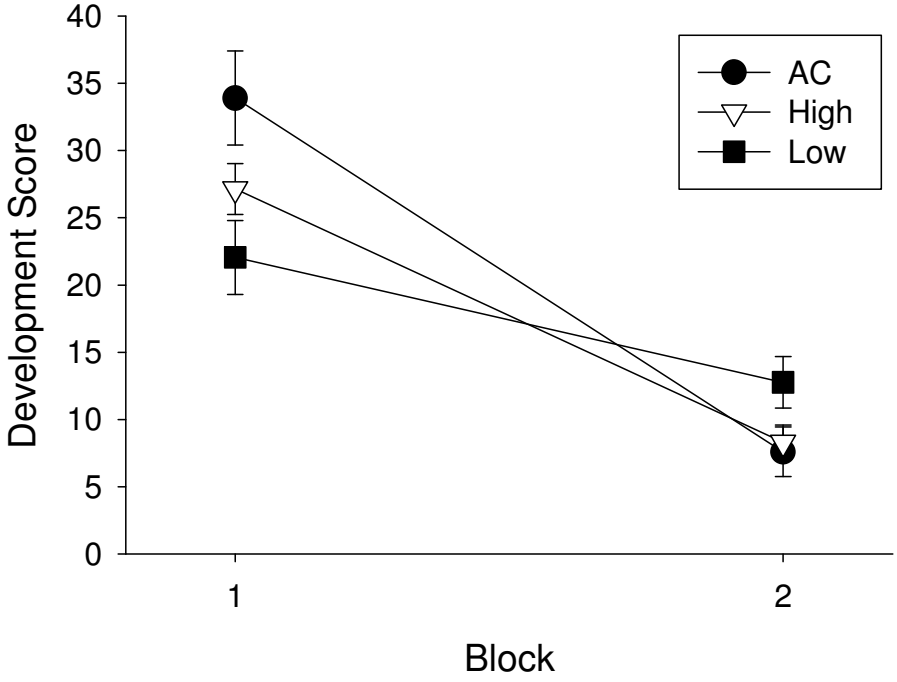


Figure 33. Experiment 2, proportion of nematodes surviving to a given day (control not included) on salt.

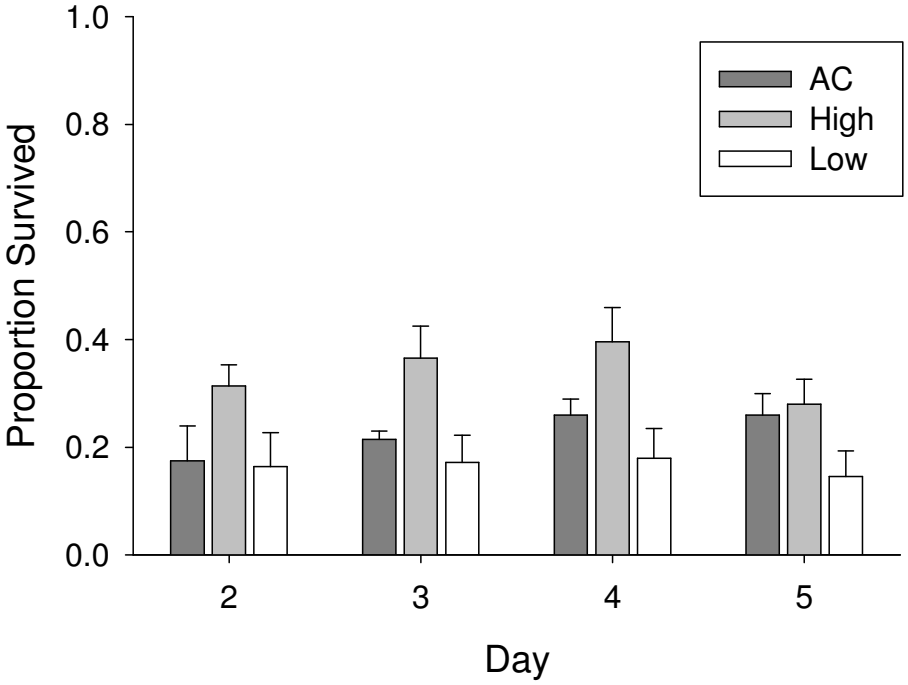


Figure 34. Experiment 2, proportion of nematodes surviving to day 3 on NGM, salt assay.

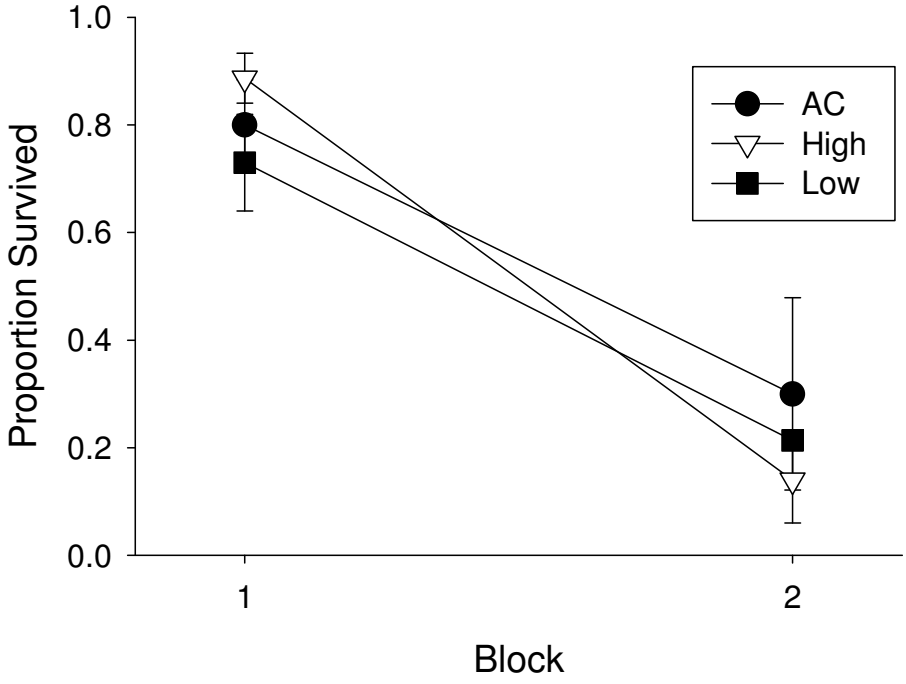


Figure 35. Experiment 2, proportion of nematodes surviving to day 3 on salt.

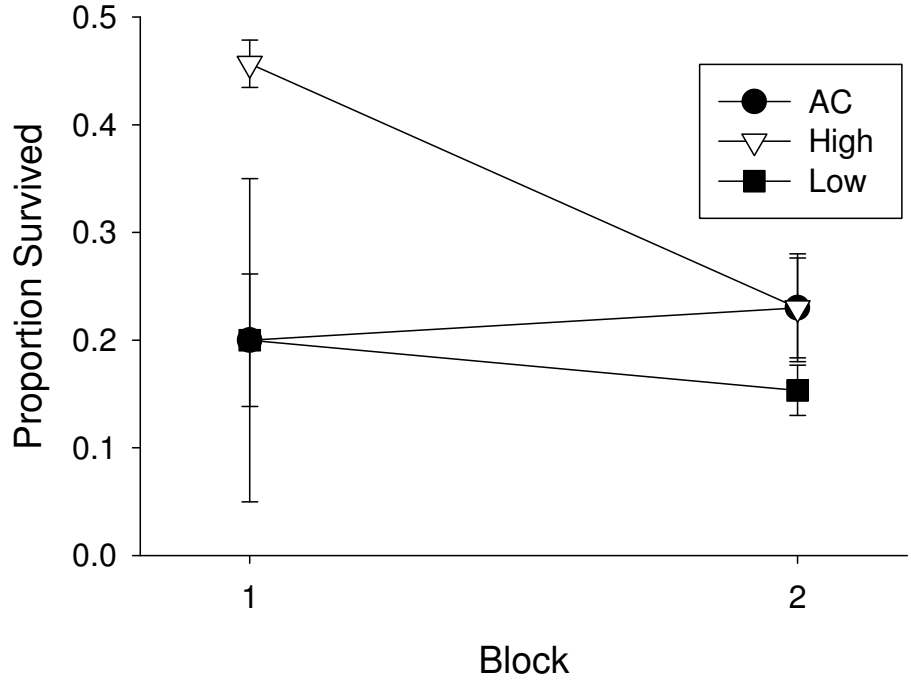


Figure 36. Experiment 2, proportion of hatched nematodes that were adults on day 3 on NGM, salt assay.

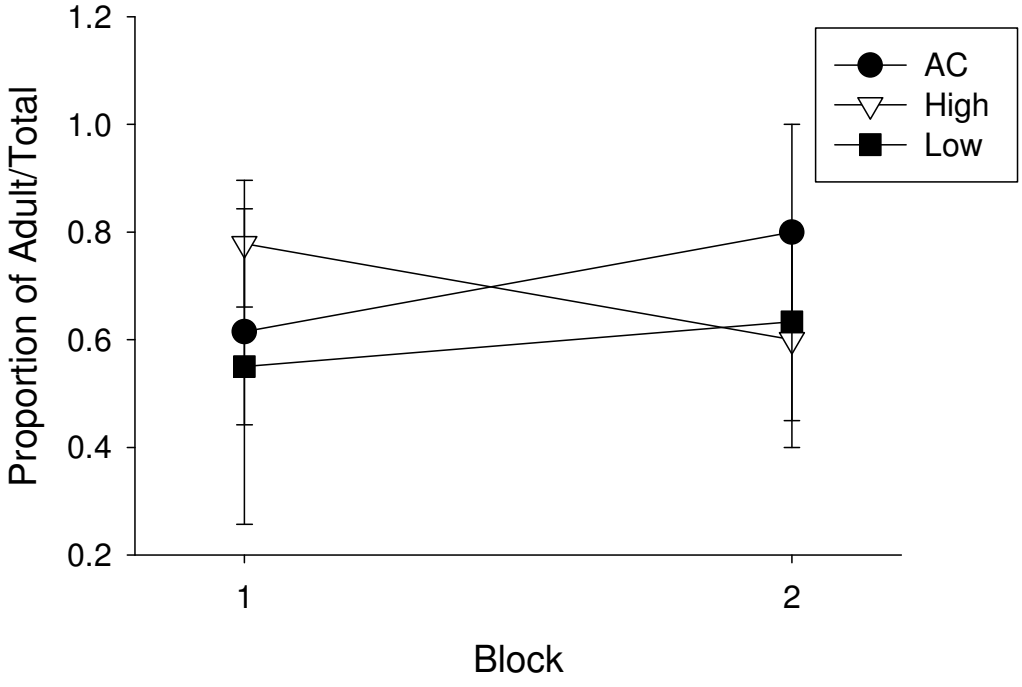


Figure 37. Experiment 2, the number of days needed to develop to adulthood on salt.

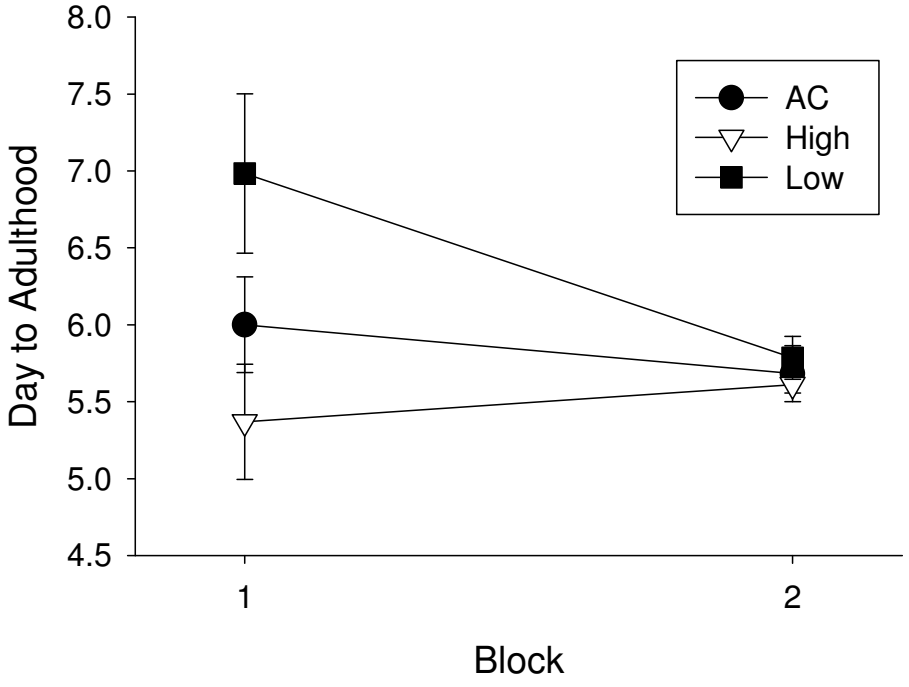


Figure 38. Experiment 2, the proportion of hatched nematodes that were adults on day 5 on salt.

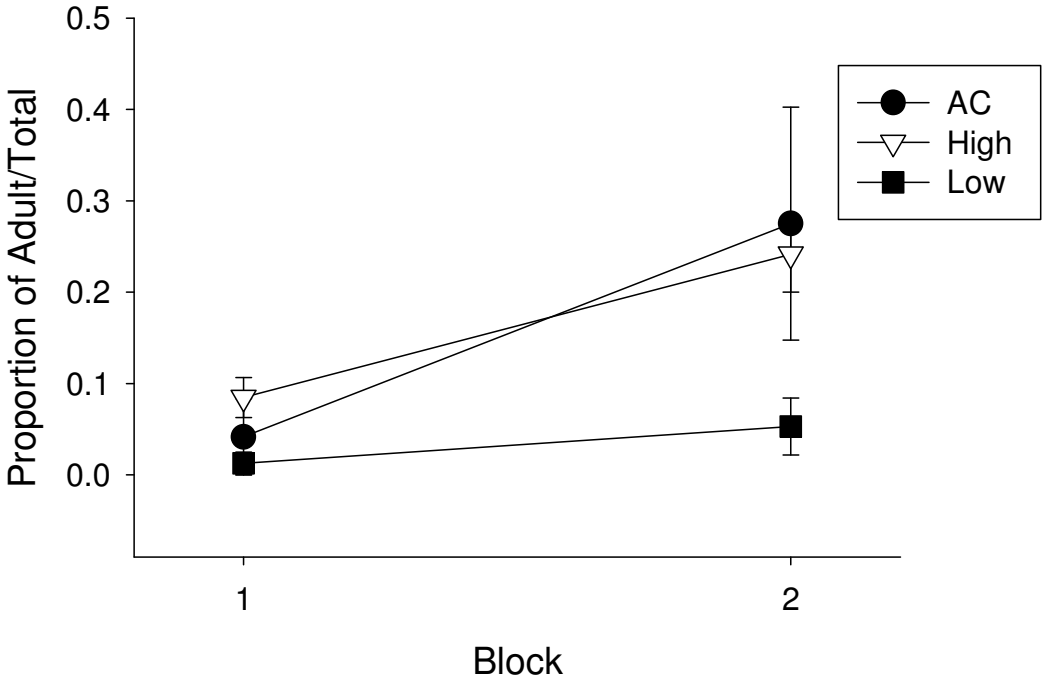


Figure 39. Experiment 2, proportion of nematodes surviving to adulthood during the entire experiment on NGM, salt assay.

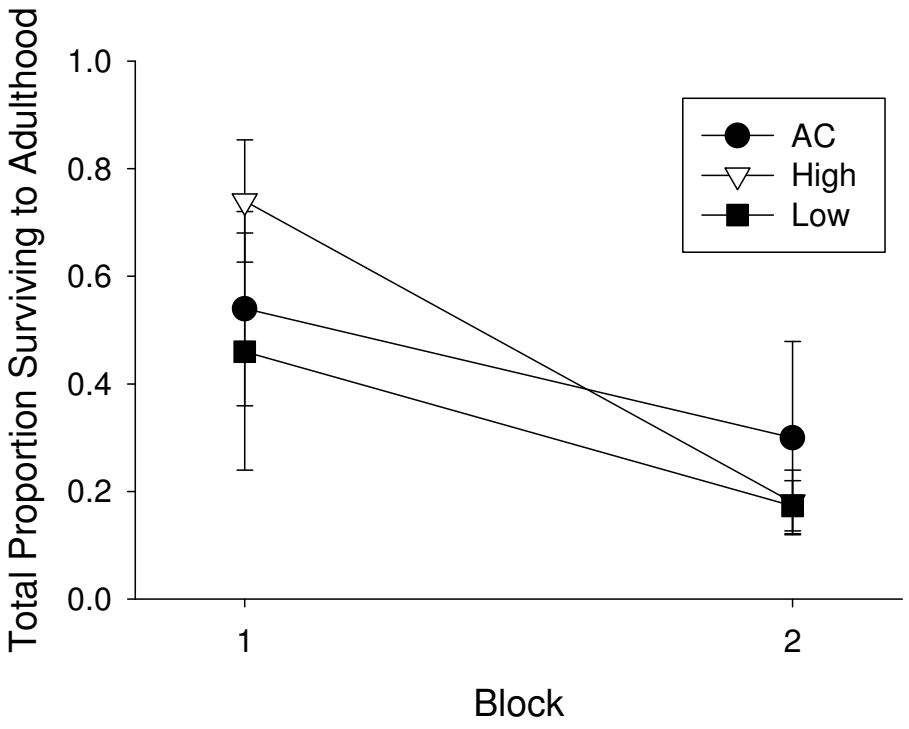


Figure 40. Experiment 2, proportion of nematodes surviving over the entire experiment on NGM and salt.

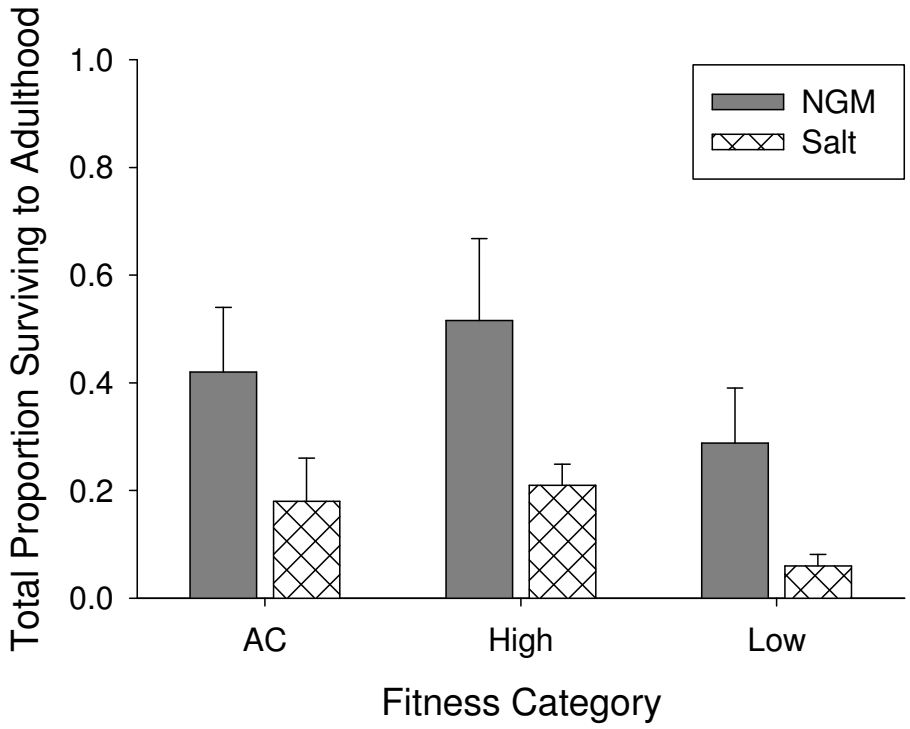


Figure 41. Experiment 2, proportion of nematodes surviving to adulthood during the entire experiment on salt.

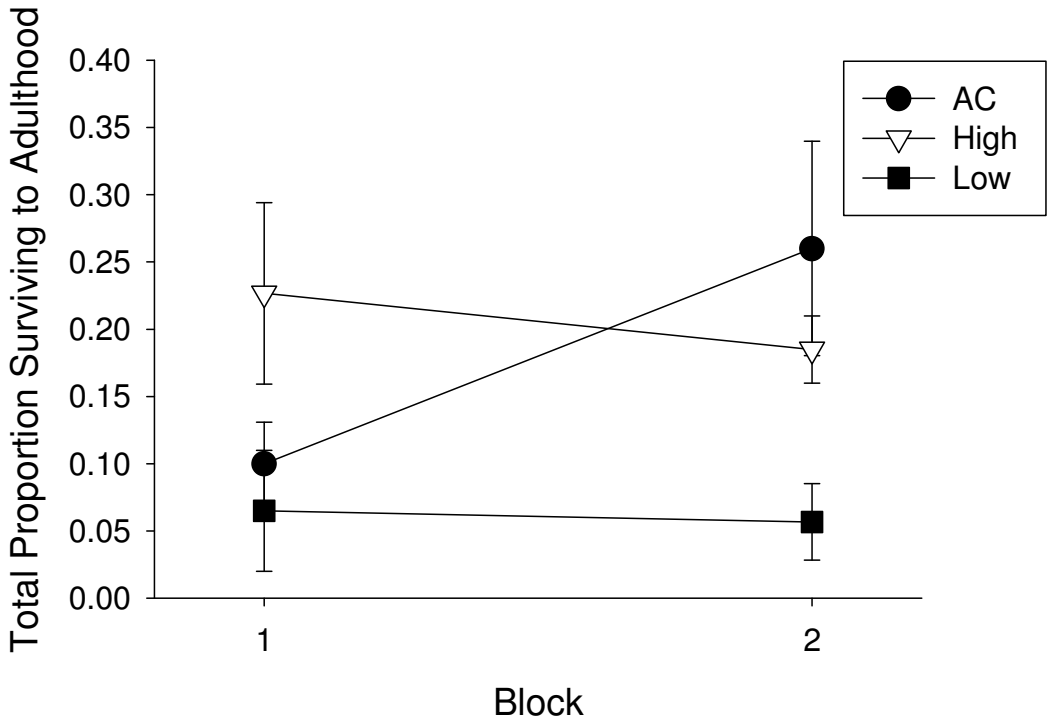


Figure 42. Experiment 2, development score on day 3 on NGM, salt assay.

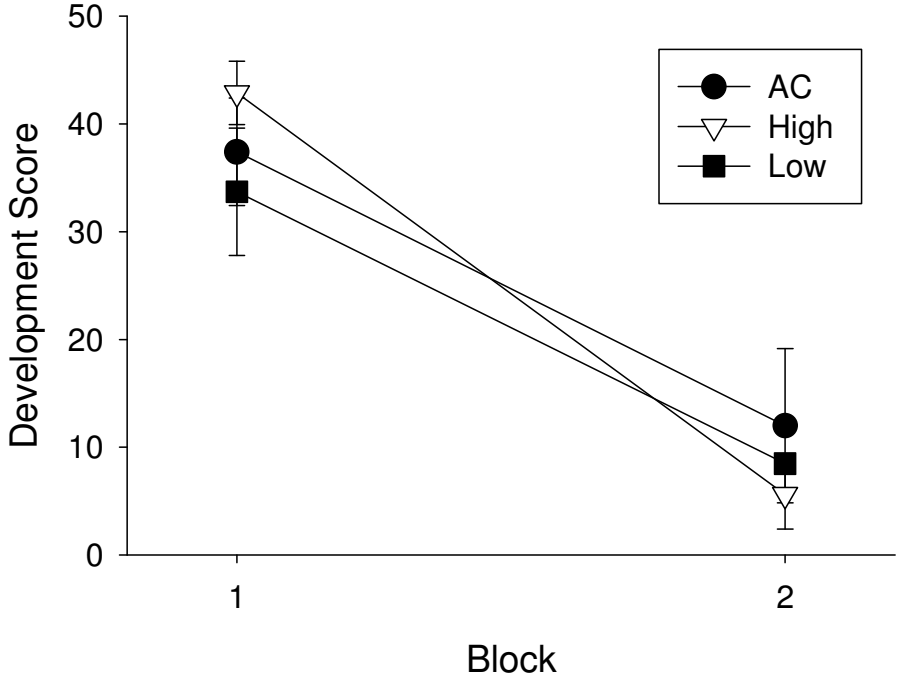


Figure 43. Experiment 2, development score over the entire experiment on salt.

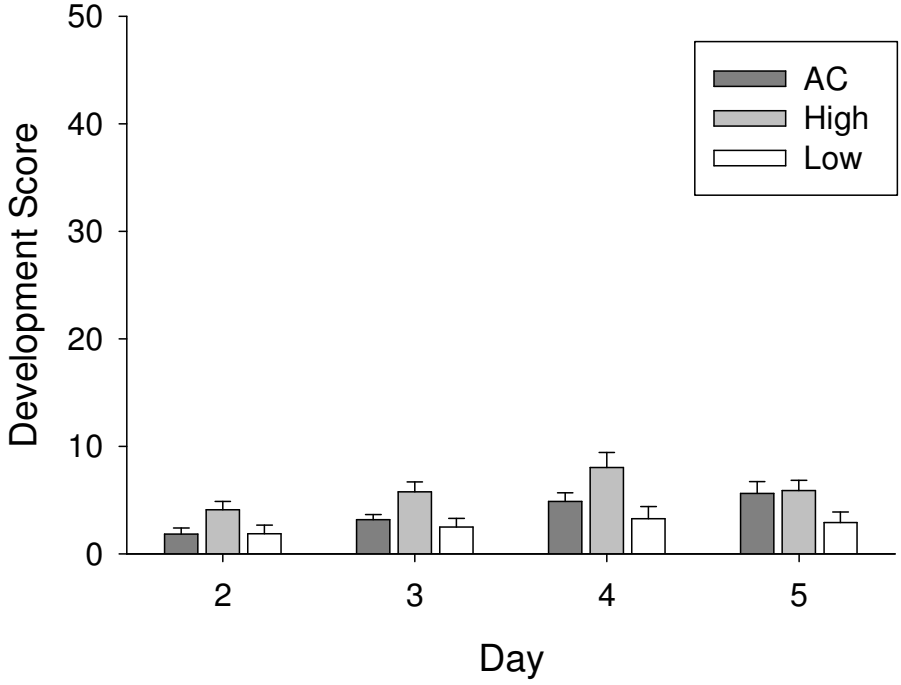
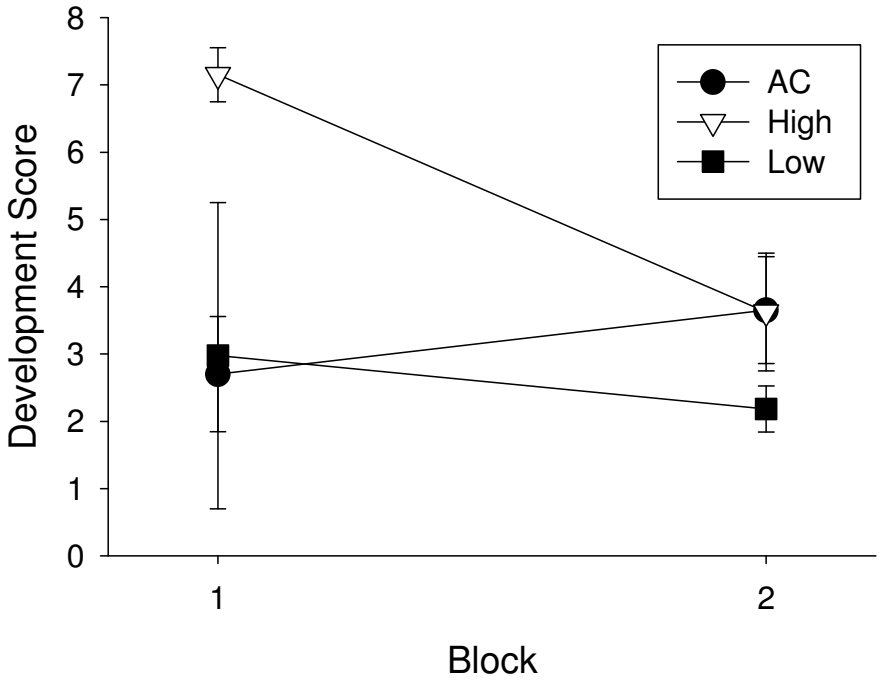


Figure 44. Experiment 2, development score on day 3 on salt.



APPENDIX B: SAS CODE

SAS code for Proc Mixed analysis, which generates estimates of among-line and within-line variance and type 3 tests of fixed effects for the MA versus ancestral control comparison. Code is for the ‘s22hs’ trait, which is the survival to 22 hours on heat stress.

```
proc mixed covtest data=seq;
class trt line rep plate;
model s22hs=trt/ddfm=kenwardroger;
random line/group=trt;
random rep(line)/group=trt;
repeated plate(rep line)/group=trt;
run;
```

SAS code to generate least square estimates of line means for each trait. Code is for the ‘s22hs’ trait, which is the survival to 22 hours on heat stress.

```
proc mixed covtest data=seq;
class trt line rep plate;
model s22hs=line/ddfm=kenwardroger;
by line;
random line;
random rep(line);
repeated plate(rep line);
lsmeans line;
ods output lsmeans=s22hsmeans;
run;
```

VITA

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Education:

Masters of Science, Biology
Eastern Washington University (EWU) – Cheney, WA
2010 – 2012

Bachelors of Science, Biology (Research Option)
Minor, Philosophy
Concentration, Environmental Studies
Gonzaga University – Spokane, WA
2005 - 2009

Research Experience:

Graduate Research, Department of Biology, Eastern Washington University, Cheney, WA. Fall 2010 – Spring 2012, PI: Joanna Joyner-Matos
Stress resistance in nematodes (*Caenorhabditis elegans*).

Lab Technician, Department of Biology, Texas A & M University, College Station, TX. Summer 2009 - Winter 2009, PI: Gil Rosenthal

Undergraduate Research, Department of Biology, Gonzaga University, Spokane, WA. Spring 2008 – Spring 2009, PI: Brook Swanson
Morphological, biomechanical, and histological characterization of stomatopod crustaceans.

Grants:

Sigma Xi Grants-in-Aid of Research	Fall 2011
Eastern Washington Graduate Student Mini Grant ($500)	Fall 2011
Eastern Washington Graduate Fellowship ($9000/year)	2011 and 2012
Howard Hughes Medical Institute Research Grant ($750)	Fall 2008

Professional Presentations:

"Relationship Between Mutation Load and Stress Resistance in *C. elegans*" (Poster), Society for the Study of Evolution (SSE), Norman, OK, 2011

Morphological and Biomechanical Variation in the Stomatopod Cuticle" (Poster), Murdoch Conference, Seattle, WA, 2008

"Morphological and Biomechanical Variation in the Stomatopod Cuticle" (Talk), Society of Integrative and Comparative Biology, Boston, MA, 2008

Published Abstracts:

Andrew, J.R., George, M., Patek, S.N., Swanson, B.O. 2009. Morphological and Biomechanical Variation in the Stomatopod Cuticle. *Society of Integrative and Comparative Biology.* 48.1

Teaching Experience:

Undergraduate research mentorship:

Laura C. Bean, Fall 2010 - 2012, co-author on SSE poster

Veniel Garza, Spring 2011 – 2012, lead author on EWU symposium poster

Human Anatomy and Physiology Series (Biology 232 – 234) – Eastern Washington University, Fall 2010 – 2012

Diversity of Life Lab (Biology 101) – Gonzaga University, Fall 2007

Cell Biology Lab (Biology 201) – Gonzaga University, Fall 2008

Coursework:

Courses taken at EWU: Biological Research Methods I & II, Biochemistry, Bioinformatics, Ornithology, Biology of Symbiosis, Neurobiology, Current topics in Microbiology, Current topics in Ecology, Current topics in Physiology

Upper Division Courses taken at Gonzaga University: Community Ecology, Research in Comparative Biomechanics, Conservation Biology, Intro to Bioanalytical Chemistry, Comparative Physiology, Behavioral Ecology, Biochemistry

Additional Work Experience:

United States Census Bureau, Portland, OR	Summer 2010
Town and Country Animal Hospital, Portland, OR	2001 – 2005
Starbucks, Spokane, WA	2007 – 2008

Community Involvement:

Student Representative to EWU Graduate Affairs Council	2011 – 2012
Biology Student Organization, EWU	2010 – 2012
Vice-President (2011 School Year)	
Spokane River Cleanup, Spokane, WA	2005 – 2011
Gonzaga Environmental Organization, Gonzaga University	2005 – 2009
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References:

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