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Field measurements of genotype by environment interaction for fitness caused by spontaneous mutations in *Arabidopsis thaliana*

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As the ultimate source of genetic diversity, spontaneous mutation is critical to the evolutionary process. The fitness effects of spontaneous mutations are almost always studied under controlled laboratory conditions rather than under the evolutionarily relevant conditions of the field. Of particular interest is the conditionality of new mutations—that is, is a new mutation harmful regardless of the environment in which it is found? In other words, what is the extent of genotype–environment interaction for spontaneous mutations? We studied the fitness effects of 25 generations of accumulated spontaneous mutations in *Arabidopsis thaliana* in two geographically widely separated field environments, in Michigan and Virginia. At both sites, mean total fitness of mutation accumulation lines exceeded that of the ancestors, contrary to the expected decrease in the mean due to new mutations but in accord with prior work on these MA lines. We observed genotype–environment interactions in the fitness effects of new mutations, such that the effects of mutations in Michigan were a poor predictor of their effects in Virginia and vice versa. In particular, mutational variance for fitness was much larger in Virginia compared to Michigan. This strong genotype–environment interaction would increase the amount of genetic variation maintained by mutation-selection balance.

KEY WORDS: **Crossing GEI, mutation accumulation, mutation-selection balance, variance GEI.**

Spontaneous mutation contributes to many fundamental processes in evolution. Mutations are important for the maintenance of genetic variation for quantitative traits, enabling populations to respond to natural selection (Houle et al. 1996). In addition, the genetic load caused by slightly deleterious mutations may favor the evolution of sexual reproduction (Keightley and Eyre-Walker 2000; Almbro and Simmons 2014) or threaten the persistence of small populations (Lande 1994; Lynch et al. 1995). Mutational pressure may also be involved in the evolution of senescence and thus life-history evolution more generally (Partridge and Barton 1993). Many recent laboratory studies have addressed the rate of mutation (e.g., Ness et al. 2012; Jiang et al. 2014), the distribution of mutational effects on fitness (e.g., Vale et al. 2012), as well as the extent to which the fitness effects of new mutations depend on

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the environment (e.g., Wang et al. 2009; Lalić et al. 2011; Hietpas et al. 2013; Latta IV et al. 2015). However, little is known about the effects of spontaneous mutations in field environments.

Laboratory studies have found that stress may increase the rate of mutation (Jiang et al. 2014), and either enhance or ameliorate the negative fitness impacts of harmful mutations (reviewed in Agrawal and Whitlock 2010); thus, the deleterious effects of mutations may vary with the environment. Variation in the environment is known to affect both the magnitude and direction of mutational effects measured in the laboratory (e.g., Fry et al. 1996; Kishony and Leibler 2003; Baer et al. 2006; Xu 2004; Vale et al. 2012; Latta IV et al. 2015). When the fitnesses of different mutant genotypes change relative to one another across environments, there is genotype by environment interaction (GEI) for the new mutations. We can consider two kinds of GEI, not mutually exclusive: crossing GEI and variance GEI, both of which have consequences for the outcome of selection.

Crossing GEI occurs when the rank order of genotype fitness changes across environments; that is, the most fit mutant genotype in one environment is not necessarily the most fit in another environment. This will favor different mutations in different environments, allowing for the maintenance of genetic variation for fitness among populations. In the case of a conditionally deleterious mutation that is neutral or beneficial in some environments, this crossing GEI will reduce the mutation's among-population average selection coefficient, resulting in a higher expected equilibrium allele frequency and thus contributing to the maintenance of genetic variation through mutation-selection balance (Gillespie and Turelli 1989). Crossing GEI may also be costly; the higher load of conditionally deleterious mutations of a habitat generalist may harm competitive ability versus a habitat specialist (Kawecki 1994).

Variance GEI does not require changes in rank order fitness but rather a change in the amount of genetic variance expressed in different environments (Latta IV et al. 2015). For example, if there is high genetic variance for fitness among different mutations in some environments but little genetic variance in other environments, this reduces the overall strength of selection on these new mutations. As a result, even if crossing GEI is absent, variance GEI for new mutations may contribute to the persistence of those mutations among populations under mutation-selection balance. Thus, it is important to quantify mutational effects under multiple field environments, to determine if GEI is common for new mutations, especially for generalization to natural populations.

The mutation accumulation (MA) approach is commonly employed to estimate spontaneous mutational rates and effects (Mukai 1964; Lynch et al. 1999; Bataillon 2003; Eyre-Walker and Keightley 2007). MA designs relax selection for many generations, usually starting from a genetically homogeneous base so that all variation is due to new mutation, and reducing the effects of selection by minimizing population size to a single individual, allowing genetic drift to dominate (e.g., Shaw et al. 2000). Fitness assays then are expected to detect an increase in among-line genetic variance and a decrease in mean fitness due to deleterious mutations. Although it is not practical to conduct MA fitness assays of many model organisms under natural conditions (e.g., *Drosophila* spp. or *Caenorhabditis elegans*), it is feasible to conduct studies in a field setting with a sessile and relatively large species such as the plant species *Arabidopsis thaliana*.

We measured the effects of mutations and GEI for new mutation in the field on the *A. thaliana* MA lines initiated by Shaw et al. (2000). Only two studies that we are aware of have attempted to measure MA under field conditions (Roles and Conner 2008; Rutter et al. 2010), but neither study encompassed the spatial range of environments and life-history phenotypes explored here. We expand upon that work, exploring GEI for new mutations in two field sites and measuring fitness from germination through seed production. Prior laboratory studies of GEI in these MA lines have manipulated a single environmental variable and failed to detect GEI for new mutations (Chang and Shaw 2003; Kavanaugh and Shaw 2005); however, field habitats will rarely vary in only a single aspect. In this study, the use of two field sites, which differ in many environmental variables, better reflects the differences between two natural habitats. In addition, we study fitness components from germination through fruit production, allowing us to estimate mutational effects on very early stages of life history that strongly influence later survivorship and reproduction (Silvertown et al. 1993; Crone 2001; Metcalf and Mitchell-Olds 2009) but are not accounted for when seedlings are grown in the greenhouse and then transplanted outside.

Materials and Methods

OVERVIEW

Arabidopsis thaliana fills many of the ideal requirements for an organism in which to study spontaneous mutation: short life cycle, high fecundity, and reproduction by selfing. In addition, it is possible to maintain viable seeds for long periods, allowing us to directly compare fitness of the founding populations to their MA descendants with newly accumulated mutations. Native to Eurasia, *A. thaliana* is widely distributed across the continent (Ratcliffe 1965) and has now invaded many other areas, including North America. Accessions collected from the midwestern United States are genetically similar to Col-0 and other accessions from northern Europe (Nordborg et al. 2005).

MUTATION ACCUMULATION

The MA lines were developed and maintained in the laboratory of R. G. Shaw at the University of Minnesota as described in Shaw

et al. (2000). Shaw propagated 120 MA lines from the seeds of a single individual of the Columbia accession. The founder was highly homozygous, so any average differences between MA lines are due only to new mutations (alterations to the sequence or heritable epigenetic changes). The lines were maintained in the greenhouse by single-seed descent ($N_e = 1$), which reduces selection and maximizes genetic drift. The founder genotype was maintained as seed stored at 4° C. Generation 24 seed was supplied by R. G. Shaw and plants from these seeds were grown in the greenhouse at the University of Maryland, College Park (UMD) by CBF and MTR to produce the generation 25 seeds, as described in Rutter et al. (2010). To reduce maternal effects and create seeds of the same age for all field plantings, in 2003 CBF and MTR generated five replicate sublines from each of the 50 MA lines and each of six lines representing the ancestral premutation genotype (ancestor; Rutter et al. 2010). All seeds planted in the field experiments (representing founder lines and MA lines) were generated from these plants grown in the greenhouse in 2003 (Fig. S1). Thus, the seeds planted in this experiment were of the same age and three generations removed from stored seed. We randomly chose a subset of 50 MA lines from generation 25 for this field study so that each line could be highly replicated, given the expectation of high environmental variance. Note that for five of the 50 MA lines in this experiment, estimates of fruit production were previously reported by Rutter et al. (2012).

FIELD SITE DESIGN

The entire design was replicated at two sites, Kellogg Biological Station in Hickory Corners, Michigan (42° N, 85° W) and Blandy Experimental Farm in Boyce, Virginia (39° N, 78° W). At each site, we planted 140 replicates of each MA line (28 seeds from each of five sublines for each of 50 lines = 7000 MA individuals) and 90 (Michigan) or 84 (Virginia) replicates of each of the six founding premutation lines (540 or 504 ancestor individuals, respectively; see Supporting Information). Pots were arranged into blocks in the field, to account for variation within the field sites. A single seed was planted in a peat pot (size 4.45 cm by 5.08 cm deep), and the peat pots were transplanted in the fall into the respective field environments. The seeds had not experienced any cold stratification when they were planted into the peat pots. One week of cold stratification is commonly used to induce germination in *A. thaliana* (Nordborg and Bergelson 1999), thus this experiment relied on the natural variation of day–night temperatures at the respective sites to provide the necessary conditions for cold stratification (see below). Germination was subsequently recorded for four to six weeks post field transplant. In the spring, survival, flowering, and fruit production were recorded; entire plants were harvested upon cessation of flowering ($N = 662$ in Michigan; $N = 1704$ in Virginia). Specifics of each site are detailed below.

KELLOGG BIOLOGICAL STATION, MICHIGAN

The field site was a fenced enclosure on the site of an old agricultural field. Peat pots were filled with topsoil originally from the site. This soil was used to line an artificial pond for 10 years before being used in this experiment, which greatly reduced the viable seed bank. Blank pots containing no seeds ($N = 650$) were planted to control for natural recruitment of *A. thaliana* in the field at Michigan. The identity of pots as blank or experimental was not revealed until after all data were collected and recorded, making them a blind control. Of 650 blank pots, 15 (2.3%) germinated a seedling and six (0.9%) produced flowers, whereas 26.5% of experimental pots germinated seedlings and 9% produced flowers. Germination rate mean and variance are significantly higher in experimental pots compared to blank pots ($P < 0.001$). However, of blank pots that germinated, survival to flowering ($P > 0.6$ for mean and variance) and fruit number ($P > 0.24$ for mean and $P > 0.5$ for variance) did not differ from that of experimental pots. Given no significant differences in fitness characteristics between blank pots that germinated and experimental pots that germinated, contamination should not bias our results, although it may add noise. Seeds were placed into soil filled pots and all pots were thoroughly bottom-watered and then misted from above just before placement in the field. Blocks were planted over three days (October 25–27, 2004). Mean daily temperatures over the next month ranged from −0.6° C to 17.2° C. Pots were not watered after planting in the field. The first rain fell two days after planting was completed.

BLANDY EXPERIMENTAL FARM, VIRGINIA

Peat pots were filled with Sunshine # 3 as the soil mix. *Arabidopsis thaliana* does not occur naturally in the experimental plot, nor is *A. thaliana* present in the soil mix, thus no blank control pots were necessary at Virginia. Blocks were planted over two days (October 30–31, 2004). Planting was slightly later in Virginia than in Michigan because of the later onset of cold temperatures at the lower latitude. Mean daily temperatures over the next month ranged from 1.6° C to 18.3° C.

DATA PREPARATION

Four response variables (fitness components) were calculated for analysis. Germination and survival to flowering were binary variables, coded as presence/absence. Germination included all seeds planted whereas survival to flowering included only seeds that germinated. Fruit number only included seedlings that survived to flowering. Raw total fitness was the product of germination, survival to flowering, and fruit number, that is, total fruit number produced by each planted seed, including zeros for plants that did not germinate or survive to flower. Although these raw variables are multiplicative, each was transformed during separate univariate analysis (germination and survival via a logit transformation

and fruit number and total fitness via ln transformation), which changes the relationship among variables. Thus, statistical inference is based upon model estimates of transformed variables but raw data are presented to illustrate patterns, where appropriate.

Evidence of the effects of accumulated mutations in this design comes from (1) a change in mean fitness between the ancestor (generation 0) and the MA lines (generation 25), (2) the presence of significant among-line (genetic) variance of MA lines due to new mutations within environments (V_L), and (3) a significant GEI. The ancestor is expected to have no genetic variance because it was derived from a single highly homozygous individual, which was confirmed by screening molecular genetic variation (Shaw et al. 2000). Any observed V_L among ancestors may derive from another source, such as epigenetic variation.

All analyses were performed in R version 3.2.2 (R Core Team 2015). The response variables consist of counts (fruit number and total fitness) and binary responses (germination and survival to flowering) and do not conform to a normal distribution. To account for the appropriate distributions (i.e., Poisson and categorical), we analyzed the data with univariate Bayesian Markov chain Monte Carlo generalized linear mixed models (MCMCglmm) using the R packages MCMCglmm (version 2.21; Hadfield 2010) and likelihood based estimation using the *glmer()* function in the lme4 library (version 1.1.9; Bates et al. 2012). In addition, our data structure contain unequal sampling of ancestor and MA lines ($N = 6$ ancestor "lines" and $N = 50$ MA lines) that caused instability when both generations were included in a single model. Thus, we evaluated ancestor and MA lines in separate models and conducted Welch *t*-tests on the model-estimated means.

The full model for ancestor lines or MA lines, with all explanatory variables, included the fixed effect of *site* (Virginia or Michigan), the random effect of *line*, the random effect of *subline* (nested within *line*), the random effect of *block* (nested within *site*), and residual error (for the formal description, see the Supporting Information). For each fitness component, we evaluated which explanatory variables to include in the final MCMCglmm model with a parametric bootstrap using the maximum-likelihood fit; this approach takes advantage of the robust estimates from MCMCglmm while accounting for the instability of such models when using a prior for random effects with low variance. Model fitting of effects allowed us to (1) evaluate the importance of covariates (such as subline and block) and (2) identify and address instability introduced by some explanatory variables. First, we constructed a series of glmer model pairs with one model including and the other excluding an explanatory variable and we calculated the observed likelihood ratio of the deviance for each model pair (see Supporting Information for details). Next, we ran 1000 replicates of the parametric bootstrap, fitting the simulated data to the model pair and constructing the likelihood ratio of the deviance for each replicate. Finally, we calculated the probability of our observed likelihood ratio as the fraction of replicates in which the simulated likelihood ratio was larger than the observed likelihood ratio. If this *P*-value was below 0.05, then we considered the focal variable to have significant explanatory power and retained the variable for the MCMCglmm analysis. Having determined the explanatory variables to retain in the model for each fitness component in the ancestor and each in the MA lines, we then ran that model using MCMCglmm and used the results to estimate our parameters of interest. We used MCMCglmm parameter estimates because these models produce more robust estimates of the random effects for non-Gaussian data (Hadfield 2010).

Within the glmer and MCMCglmm model structures, we were able to test for both types of GEI by modifying the variance structure of the model. Significant variance GEI is supported when the parametric bootstrap results indicate separate among-line variances for the two sites and can be further assessed by examining overlap of the 95% CIs from the MCMCglmm models. We tested for crossing GEI by estimating the across-site line covariance, from which we could estimate the cross-environment genetic correlation (using the *us* variance structure in MCMCglmm); a correlation significantly less than +1 is evidence of crossing GEI (Falconer 1952; Via 1984; Falconer and Mackay 1996). For some traits, the glmer and MCMCglmm model results yielded extremely wide confidence intervals on the genetic correlation (nearly −1 to +1); as a result, we further assessed the genetic correlation via a nonparametric hierarchical bootstrap of the glmer models and a Spearman's rank correlation (see Supporting Information for formal variance structure and details).

For each trait, the per-generation increase in genetic variance due to mutation (mutational variance, V_M) was calculated as $V_M = V_L/2t$, where V_L is the among-line variance and t is the number of generations of divergence (Lynch and Walsh 1998). Mutational heritability (h^2_M), which is the rate of increase in heritability due to new mutations, was calculated as $h^2_M = V_M/V_E$ (Houle et al. 1996) and the mutational coefficient of variation was calculated for the poisson variables as $CV_M = 100(e^{SD} - 1)$ for ln scale, where SD is the square root of V_M in this analysis. For each of these estimates, 95% CIs were estimated from the posterior distributions of the MCMCglmm output. Note that the non-normality of the data and the use of log or logit transformations in analysis will influence the estimates. For example, the transformations eliminate the multiplicativity of the fitness components estimated from the model outputs.

Results

MEAN AND VARIANCE ESTIMATES

The model-estimated means were significantly higher in the MA lines compared to the ancestor for all fitness components in each field site except for germination rate (Table 1; Fig. 1). In

Table 1. Raw data sample sizes, means, standard errors, and *P*-values for the comparison of MA and ancestor line means within a site for fitness components of both ancestor and MA lines.

Site	Line type	*N*	Mean	Standard error	*P*
Germination rate					
Michigan	Ancestor	540	0.27	0.02	0.738
Michigan	MA	7000	0.27	0.01	
Virginia	Ancestor	504	0.52	0.02	0.083
Virginia	MA	7000	0.53	0.01	
Survival to flowering					
Michigan	Ancestor	145	0.30	0.04	<0.001
Michigan	MA	1856	0.34	0.01	
Virginia	Ancestor	261	0.38	0.03	<0.001
Virginia	MA	3727	0.43	0.01	
Fruit number					
Michigan	Ancestor	42	5.59	0.85	0.006
Michigan	MA	620	6.95	0.45	
Virginia	Ancestor	98	154.10	13.73	0.016
Virginia	MA	1606	174.95	3.57	
Total fitness					
Michigan	Ancestor	539	0.44	0.09	<0.001
Michigan	MA	6986	0.62	0.05	
Virginia	Ancestor	504	29.96	3.80	<0.001
Virginia	MA	7000	40.14	1.20	

We report raw values here but provide significance values based on model estimates (from transformed data). Means for Michigan and Virginia are significantly different ($P < 0.002$) for germination rate, fruit number, and total fitness (germination × survival × fruit production) but not for survival to flowering ($P = 0.92$). Differences between MA and ancestor model-estimated line means were evaluated with a Welch two-sample *t*-test. In Michigan, one ancestor plant and 14 MA plants survived to flower but are missing values for fruit number and total fitness.

comparison to the ancestor, raw mean total fitness of MA lines was about 40% higher in Michigan (1.6% per generation) and 34% higher in Virginia (1.4% per generation; $P < 0.001$ for both sites), driven by increases in both survival to flowering and fruit number.

Across sites, mean values were significantly greater in Virginia than Michigan for all fitness components except survival to flowering, with the raw data reflecting a twofold difference for germination rate, a 25-fold difference in number of fruits, and about 64-fold difference for total fitness (Table 1).

The parametric bootstrap supported significant among-line variances (V_L) for germination rate ($P = 0.001$), number of fruits ($P = 0.001$), and total fitness ($P = 0.002$) in the MA lines (Table 2; see Supporting Information for details); V_L for survival to flowering was marginally significant ($P = 0.104$). Estimates of mutational variability are reported in Table 2.

GENOTYPE–ENVIRONMENT INTERACTION

We find strong evidence of GEI for germination rate, number of fruits, and total fitness, with variance GEI being the dominant pattern. The parametric bootstrap results support site-specific among-line variance estimates for all three traits (germination rate $P = 0.002$; number of fruits $P = 0.001$; total fitness $P = 0.001$; Table 2) and this is visible in reaction norm plots (Fig. 2). Due to proximity to the lower bound of zero, overlap of the 95% CIs for V_L estimates for the two sites persisted despite testing a variety of model parameters and approaches (glmer, MCMCglmm, and ADMB). We report estimates from MCMCglmm, which is considered a robust approach (Hadfield 2010); estimates from other approaches (e.g., glmer) were qualitatively similar.

We also found evidence for crossing GEI for total fitness as seen in the reaction norm plots (Fig. 2) and the estimates of the cross-environment correlation. The latter were near zero using all four methods (Table 3; Fig. 3), suggesting little relationship in performance between Michigan and Virginia. The cross-environment correlations for the two fitness components with significant among-line variance were also near zero except for the glmer estimate for germination rate and both Spearman estimates, with the latter being significantly positive (Table 3).

Our estimates of mutational variance, heritability, and coefficient of variation (Table 2) for Virginia are consistently four- to fivefold higher than for Michigan, reflecting the presence of variance GEI. This suggests that there is much more potential for a response to selection on mutations affecting germination rate, number of fruits, or total fitness in Virginia than in Michigan. Combined with the presence of crossing GEI, these results

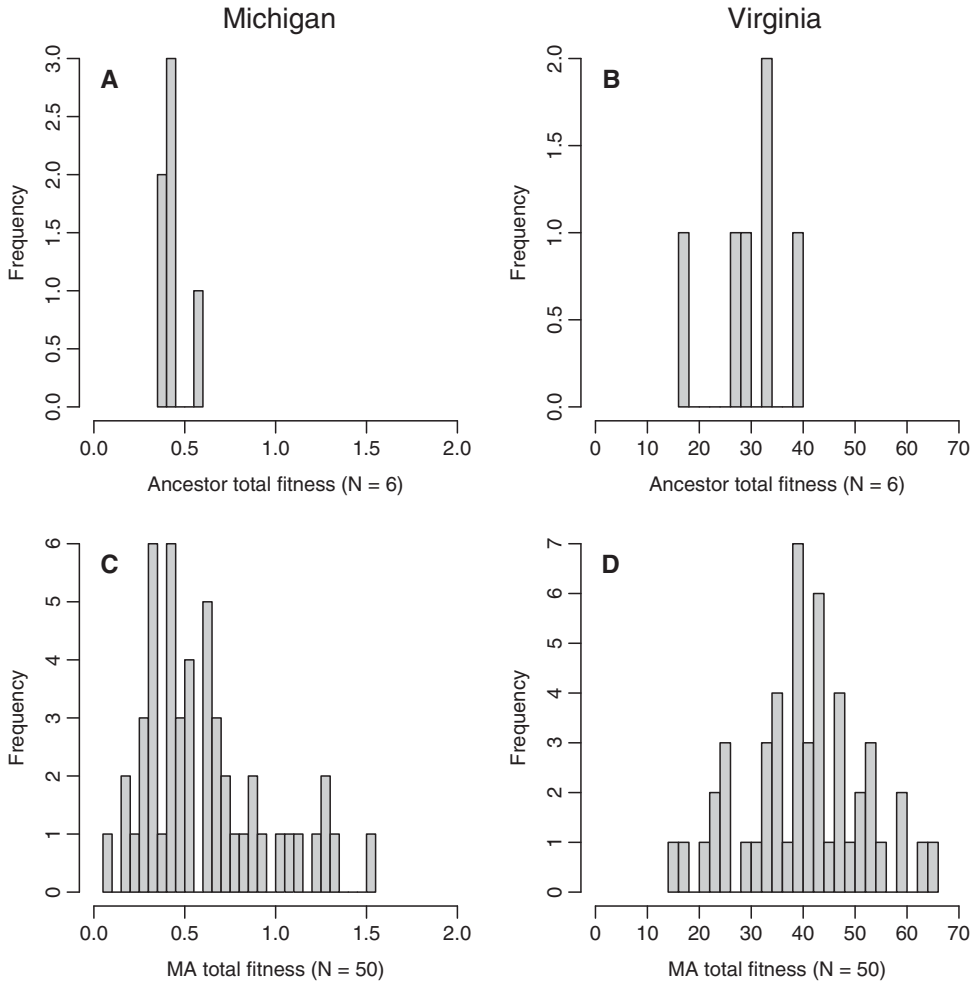

Figure 1. Distribution of raw line means. (A) Michigan ancestor lines, (B) Virginia ancestor lines, (C) Michigan mutation accumulation (MA) lines, (D) Virginia MA lines. Note that the range of the *x*-axis differs between Michigan and Virginia.

support substantial spatial variation in the phenotypic expression of mutational variance for fitness.

Discussion

EFFECTS OF MUTATION ON MEAN AND VARIANCE

Studies of MA have considered a broad range of taxa, including several viruses (ϕ6, Burch et al. 2007; *Tobacco etch polyvirus*, Lalić et al. 2011; ϕX174, Vale et al. 2012), several bacteria (*Escherichia coli*, Kibota and Lynch 1996; *Bacillus subtilis*, Sung et al. 2015), a protist (*Tetrahymena thermophila*, Long et al. 2013), several fungi (*Cryptococcus neoformans*, Xu 2004; *Saccharomyces cerevisiae*, Hall et al. 2008), a unicellular alga (*Chlamydomonas reinhardtii*, Morgan et al. 2014), a slime mold (*Dictyostelium discoideum*, Hall et al. 2013), several invertebrates (*Drosophila melanogaster*, Fry and Heinsohn 2002; *Caenorhabditis* spp., Baer et al. 2006; *Daphnia pulicaria*, Schaack et al. 2013), and several plant taxa (discussed below). In these studies, mean fitness declines as mutations accumulate, leading to the conclusion that new spontaneous mutations are deleterious on average. In contrast to this body of laboratory studies, we observed that the mean total fitness of the MA lines measured at both field sites has increased relative to the ancestor, indicating that mutational effects were not on average deleterious. Among MA studies of plant taxa other than *A. thaliana*, fitness declines were found in a field study of the related *Raphanus raphanistrum* (Roles and Conner 2008) and a study of two species of *Amsinckia* (Schoen 2005). However, all previous MA studies of *A. thaliana* except one (Schultz et al. 1999, with the Landsberg erecta genotype), have found no change or an increase in mean fitness due to new mutations, including studies of the same MA lines (Shaw et al. 2000; Chang and Shaw 2003; Kavanaugh and Shaw 2005; Rutter et al. 2010) and an independent experiment using the same *A. thaliana* ancestor (MacKenzie et al. 2005).

Although it is possible that additional generations of MA would lead to a significant decline in the mean, the presence

Table 2. MCMCglmm estimates and 95% confidence intervals (CI) of MA among-line variance (V_L), mutational variance ($V_M = V_L/2t$), and mutational heritability ($h^2_M = V_M/V_E$) for all fitness components.

	Michigan		Virginia	
Estimate	Mean	95% Confidence interval	Mean	95% Confidence interval
Germination rate				
V_L	0.011	(2.99×10^{-4} to 0.0343)	0.053	(8.12×10^{-4} to 0.105)
$V_M \times 10^{-3}$	0.228	(0.00598–0.687)	1.06	(0.0162–2.10)
$h^2_M \times 10^{-3}$	0.053	(0.00139–0.160)	0.246	(0.00379–0.490)
Survival to flowering				
V_L	0.032	(4.57×10^{-4} to 0.075)		
$V_M \times 10^{-3}$	0.639	(0.009–1.50)		
$h^2_M \times 10^{-3}$	0.149	(0.002–0.350)		
Fruit number				
$V_L \times 10^{-3}$	0.003	(1.01×10^{-9} to 0.0121)	0.014	(9.02×10^{-8} to 0.0297)
$V_M \times 10^{-3}$	0.062	(2.01×10^{-8} to 0.241)	0.287	(1.80×10^{-6} to 0.594)
$h^2_M \times 10^{-3}$	0.093	(2.96×10^{-8} to 0.356)	0.428	(2.77×10^{-6} to 0.895)
CV_M	0.630	(4.48×10^{-4} to 1.57)	1.63	(0.592–2.73)
Total fitness				
V_L	0.112	(4.24×10^{-10} to 0.422)	0.572	(8.25×10^{-6} to 1.12)
$V_M \times 10^{-3}$	2.23	(8.47×10^{-9} to 8.44)	11.4	(1.65×10^{-4} to 22.5)
$h^2_M \times 10^{-3}$	0.062	(2.35×10^{-10} to 0.238)	0.317	(4.38×10^{-6} to 0.618)
CV_M	3.90	(0.00143–9.62)	10.8	(3.49–17.5)

The parametric bootstrap supported separate estimates of among-line variance for germination rate ($P = 0.002$), fruit number ($P = 0.001$), and total fitness ($P = 0.001$) but only a single among-line variance across sites for survival to flowering ($P = 0.339$). Estimates are based on the MCMCglmm model fits. The mutational coefficient of variation (CV_M) is reported for the poisson variables fruit number and total fitness; a CV is unnecessary to describe the frequency distribution of the binary variables (germination). The data were transformed with a link function for analysis; all calculations were performed on the transformed data (see Supporting Information) and thus are on a different scale than the raw means reported in Table 1.

of new beneficial mutations would explain the observed phenotypic distribution. By allowing the distribution of mutational effects to span zero (in contrast to previous methods), Shaw et al. (2002) estimated that up to half of the mutations observed in their *Arabidopsis* MA lines may be beneficial. Mutations have certainly accumulated in these MA lines, supported by the observation of significant increases in among-line variance (Shaw et al. 2000; Rutter et al. 2010) and sequencing of a subset of lines (Ossowski et al. 2010). In fact, if the founder genotype is not well adapted to the assay environment, a greater proportion of mutations are expected to be beneficial (Fisher 1930; Martin and Lenormand 2006), as observed in experiments with viruses and bacteria (Burch and Chao 2000; Silander et al. 2007; Lalić et al. 2011; Perfeito et al. 2014) and recently with *A. thaliana* following ethylmethane sulfonate (EMS) mutagenesis (Stearns and Fenster 2016). The strong variance and crossing GEI between our two field sites indicates that new mutations had very different effects on fitness in the two environments. In addition, *A. thaliana* harbors substantial genetic diversity within and among natural populations (Schmid et al. 2006), with recent studies supporting local adaptation across the species' range (Fournier-Level et al. 2011; Ågren and Schemske 2012). Thus, it is clear that the Columbia accession does not represent a generalized "optimal" genotype in field environments, increasing the chances that new spontaneous mutations may be neutral or beneficial rather than harmful.

We found that environmental stress (as measured by absolute fitness) did not change the average effect of a mutation on fitness, in agreement with other studies of stress and spontaneous mutation (reviewed in Agrawal and Whitlock 2010). Absolute fitness estimates are lower in Michigan than Virginia for germination rate, number of fruits, and total fitness but the percent difference in fitness between ancestor and MA lines is similar within environments for both fitness components and total fitness (Table 1). Although the average strength of selection on new mutations did not differ between the two field sites, the among-line variance in fitness of MA lines (reflected in the coefficient of mutational variation, CV_M)—was higher in Virginia than in Michigan (Table 2, Fig. 2). Stress is usually thought to increase variance in fitness effects of new mutations (Martin and Lenormand 2006; Agrawal and Whitlock 2010; but see Kishony and Leibler 2003), rather than decrease it as observed here. Thus, despite the expectations for harsher conditions, and possibly stronger overall phenotypic selection in the field, we find that spontaneous mutations are not, on average, harmful in these field

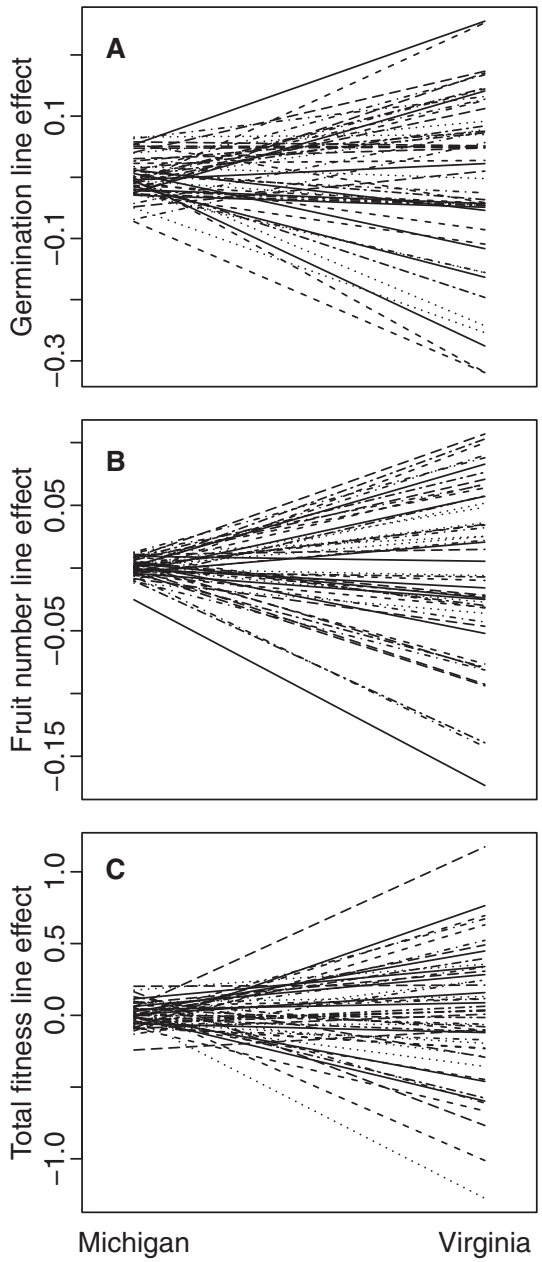

Figure 2. Reaction norms for (A) germination rate, (B) fruit number, and (C) total fitness. Each line represents a mutation accumulation line's predicted effect, minus the overall site effect, estimated from the MCMCglmm posterior predictions. Note that mixed models incorporate uncertainty in random effects parameters, producing shrinkage relative to estimates from raw data.

environments and are thus likely to contribute to standing genetic variation.

GEI FOR NEW MUTATIONS

GEI for new mutations has been studied in a variety of organisms and laboratory environments with mixed results in most systems. Support for GEI is found in four of five studies in *D. melanogaster* (Kondrashov and Houle 1994; Fry et al. 1996; Fernández and López-Fanjul 1997; Shabalina et al. 1997; but see Fry and Heinsohn 2002) and one of three studies in *C. elegans* (Matsuba et al. 2013; but see Vassilieva et al. 2000; Baer et al. 2006). In addition, a single study in *Daphnia pulex* (Latta IV et al. 2015) and several studies of microbial and viral mutants (Szafraniec et al. 2001; Lalić et al. 2011; Vale et al. 2012) have detected GEI. These studies encompass a broad range of taxa and life-history strategies—although notably all were performed in the laboratory. Taken together, the available evidence suggests that GEI for new mutations is common but not ubiquitous, calling into question the assumption that the effects of mutations are uniform.

Our results suggest little to no relationship between relative fitness in the two field environments—in contrast to prior laboratory studies of GEI in these *A. thaliana* MA lines that estimated genetic correlations overlapping one (Chang and Shaw 2003; Kavanaugh and Shaw 2005). Perhaps the factors most likely to explain mutational GEI in this study versus previous work with *A. thaliana* are the higher dimensionality of the field environment and the inclusion of very early life history (germination rate). Both of the prior laboratory studies were conducted under relatively benign conditions that differed in a single manipulated variable and neither included germination rate as a fitness component. Our study included early life history in two field environments that differ in multiple variables and cover a large range of environmental variation. The effects of spatial and temporal variation in field environments on the expression of mutational GEI is supported by Rutter et al.'s (2012) report of significant crossing GEI in the comparison of performance by a set of five MA lines across six separate field assays (including the results reported here for those five lines).

Mutational effects on fitness in the field result from the simultaneous interaction of many environmental variables and may not be predictable on the basis of a single variable, reducing the applicability of laboratory studies to field environments. The few laboratory studies that have manipulated multiple environmental variables simultaneously have all found evidence of GEI (Kondrashov and Houle 1994; Xu 2004; Lalić et al. 2011; Vale et al. 2012). In contrast to laboratory studies, field studies must contend with a large environmental variance component with the potential to reduce the power to detect the effects of new mutations. The field sites studied here differ in environmental factors (e.g., precipitation, day length, soil type and moistures, local flora and fauna) some of which have substantial temporal variation within sites; thus, without replication in time or space we cannot relate fitness differences to any given factor. Field studies that intentionally vary environmental factors (e.g., use shade cloth to reduce light, apply fertilizer to control nutrient availability) may enhance the signal for a specific environmental factor while maintaining the multidimensionality and evolutionary relevance of the field environment.

Table 3. Cross-site MA line correlation estimates for germination rate, fruit number, and total fitness.

Trait	MCMCglmm (95% CI)	glmer (95% CI)	HB (2.5%, 97.5%)	Spearman's rho (P-value)
Germination rate	0.15 (−0.88, 0.98)	1.00 (−1.00, 1.00)	0.04 (−0.37, 0.47)	0.33 ($P = 0.02$)
Fruit number	0.08 (−0.98, 0.99)	0.02 (−0.27, 0.31)	0.02 (−0.35, 0.38)	0.41 ($P = 0.003$)
Total fitness	−0.03 (−1.00, 0.99)	0.10 (−0.18, 0.37)	0.08 (−0.21, 0.37)	0.009 ($P = 0.95$)

MCMCglmm correlation estimates, glmer correlation estimates, nonparametric hierarchical bootstrap (HB) quantiles glmer correlation estimates, and Spearman's rank correlation test (for line means estimated from MCMCglmm model fits). Note that estimates from MCMCglmm models included subline whereas estimates from glmer and HB models did not include subline due to lack of convergence resulting from lack of subline variance in Michigan.

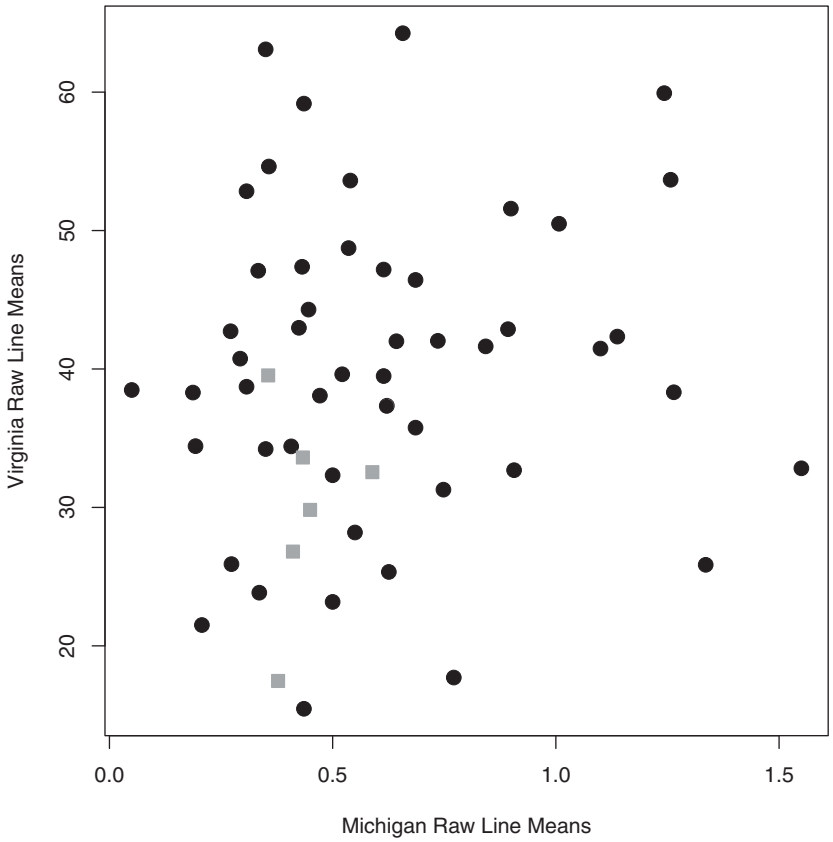

Figure 3. Scatterplot of ancestor (■) and mutation accumulation (MA, ●) raw line means for total fitness at each site. Correlation coefficients are reported in Table 3.

Such field manipulations should be relatively straightforward for plant species such as *A. thaliana*. An additional complicating factor for field studies is the often very large interannual variation in environmental variables, which means that field studies should ideally be replicated across time. Rutter et al.'s (2012)) comparison of five genotypes across six assays encompassing two years and two field environments demonstrates this well—the least fit line in one spring had the second highest fitness in the next spring, at the same field site. Despite the difficulties of studying mutations with field approaches, to understand the impact of mutation with regard to evolutionary processes such as the maintenance of genetic variation, it is essential that we study how mutations are expressed in natural populations. Future studies should ideally be replicated across space, as here, but also over time.

CONCLUSIONS: EVOLUTIONARY CONSEQUENCES

Our study suggests that the effects of spontaneous mutations on fitness are dependent on the field environment in which the mutations are expressed with stress decreasing the variance in fitness. However, we encountered statistical challenges estimating the relevant variances, despite large sample sizes; this suggests that further development of statistical methods will be important in future studies of mutational variance. The results of this study also support the idea that neutral or beneficial mutations may

occur at an appreciable frequency, much more often than previously thought. These results, combined with those of other studies showing GEI for spontaneous mutation, suggest that much of the standing variation due to mutation in populations is not deleterious in all environments, and is therefore available for adaptive evolution.

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1: Graphical representation of lineages planted in this experiment.
Table S1: Description of dataset.
Table S2: Variance structures of random effects employed during analysis with MCMCglmm, using the random effect of LINE as an example.
Table S3: Models constructed for the parametric bootstrap.
Table S4: Observed likelihood ratio (Obs LR), *P*-value, and number of warnings resulting from the parametric bootstrap for each pair of models tested (see Table S3, for mutation accumulation (MA) and ancestor lines considered separately).
Table S5: Line means and standard errors for total fitness at each site, estimated from the raw data.