Appendix 1

Rationale for 45 μM zebularine treatment: pilot demethylation experiment

A treatment level of 45μM zebularine was chosen to maximize demethylation while minimizing growth disruptions: in a pilot experiment we germinated *P. persicaria* plants on 0.8% agar plates containing all concentrations of zebularine between 5μM and 75μM (in 5μM increments), a range proven effective in studies of *Medicago truncatula* and *A. thaliana* [1], and *Taraxacum officinale* [2]. Concentrations higher than 50μM stunted seedling growth (J. Herman and S. Sultan, unpublished data). When transplanted into soil and grown to maturity, plants that had been treated with 45μM zebularine developed normally and reached equivalent final biomass compared to control plants (*t*-test, P=0.44, n=5 per treatment). A similar concentration of zebularine (40 μM) reduced global 5-methyldeoxycytidine levels by 15-18% in *M. truncatula* and *A. thaliana*; these reductions were transient, with methylation levels in zebularine-treated plants returning to normal levels after several weeks' growth in the absence of zebularine [1].

Rationale for omitting significance testing of variance components in linear mixed-effect models

We did not test the significance of the random effect of genetic line and its interactions because there is no generally accepted method to do so for complex mixed models involving high-order random effects [3]. The most common approach to significance testing of random effects in linear mixed models uses the likelihood ratio χ^2 test, yet this test is well known to be overly conservative because the distribution of the test statistic does not conform to a single χ^2 distribution, but instead conforms to a mixture of χ^2 distributions with different degrees of freedom depending on the difference in parameters in the models under comparison [4-7]. Currently there is no well-established method for determining the correct mixture of χ^2 distributions for correctly testing the significance of high-order random effects such as three-way interactions (see discussion and references in [3, 5, 7]). A straightforward alternative is to determine whether the random effects account for substantial trait variation (e.g., [8-10]).

Literature cited

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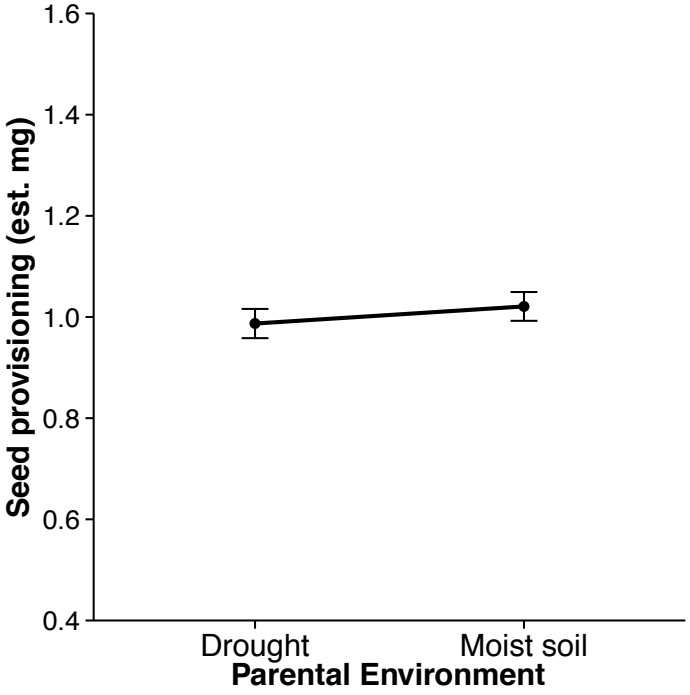
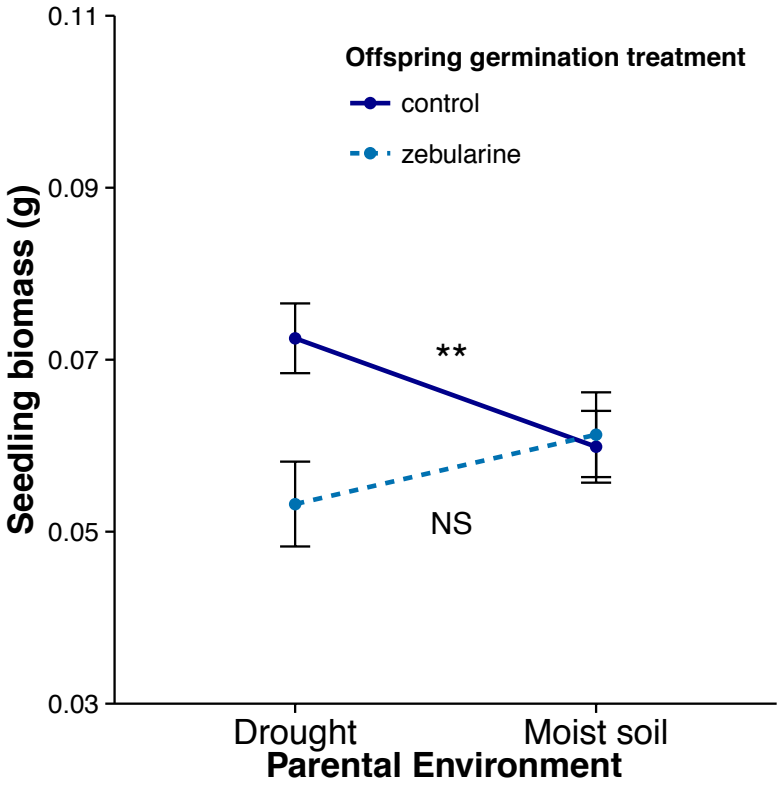
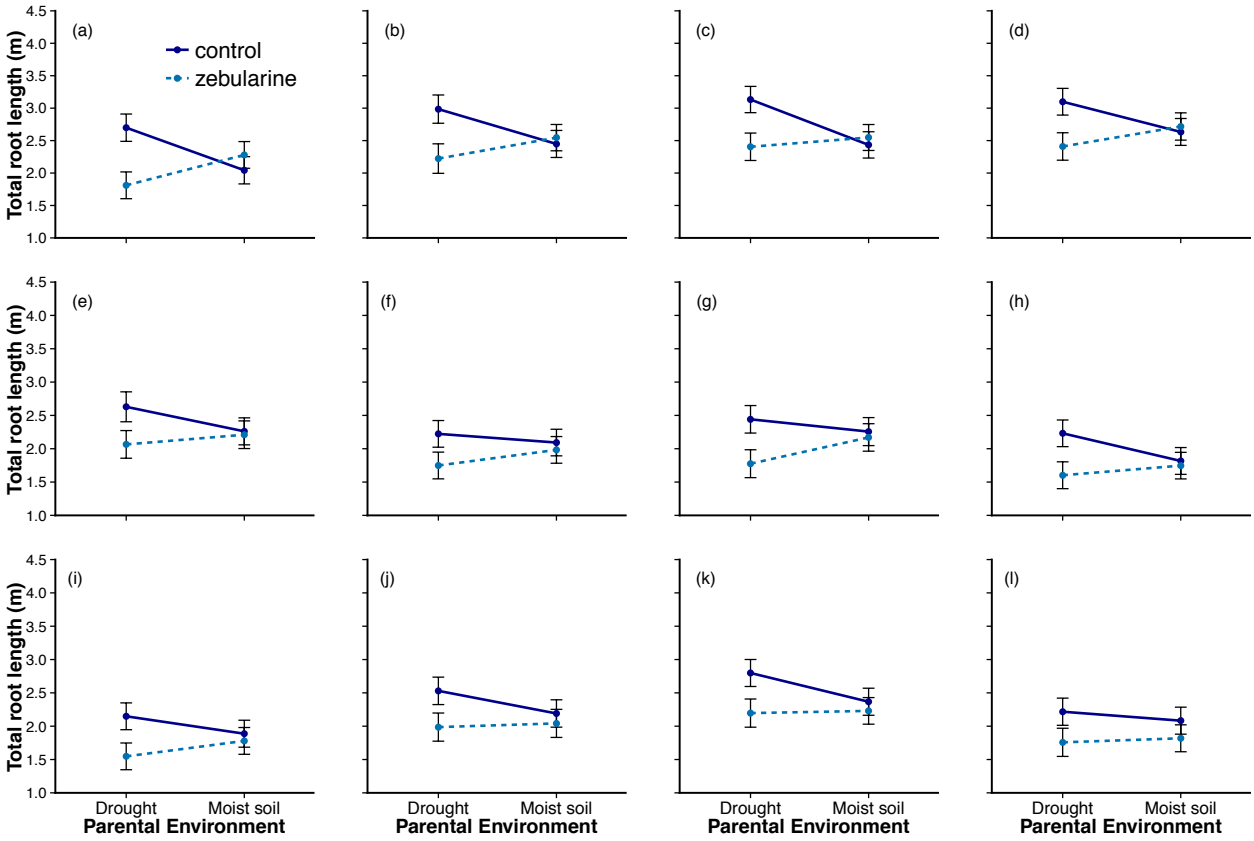
Supplemental Figure 1. Parental drought vs. moist-soil environment did not significantly influence offspring seed provisioning (means ± SE).



Supplemental Figure 2. Effects of parental environment and offspring demethylation on the biomass (means ± SE) of 114 seedlings that germinated on the same day are consistent with analyses of the full biomass dataset (N=180), ruling out the possibility that the lower biomass of offspring of drought-stressed parents (compared to offspring of well-watered parents) resulted from slower germination and consequently longer exposure to zebularine. Asterisks indicate significance of the parental drought effect (one-way ANOVA separately testing the effect of parental environment on control-germinated and zebularine-germinated seedlings, ** $P < 0.01$, NS, non-significant, see *Results* for details).



Supplemental Figure 3. Genetic variation for the effect of parental drought on total seedling root length and its alteration by demethylation, reflecting the interaction between *genetic line*, *parental environment*, and zebularine vs. control *germination treatment* in the linear mixed-effect model (see Table 1). Each plot displays means ± SE for one genetic line, presented in the same order as in Figure 4.



Supplemental Figure 4. Genetic variation for the effect of parental drought on seedling leaf area and its alteration by demethylation, reflecting the interaction between *genetic line*, *parental environment*, and zebularine vs. control *germination treatment* in the linear mixed-effect model (see Table 1). Each plot displays means ± SE for one genetic line, presented in the same order as in Figure 3.