SUPPORTING INFORMATION:
Variation within and between populations of *Boechera stricta* in the the allocation of methionine- vs. branched chain amino acid-derived glucosinolates and levels of insect herbivory

INTRODUCTION

Variation in glucosinolates and insect feeding between and within populations

While much can be learned about ecologically important variation by QTL mapping, one drawback is that usually only the variation of the two parental genotypes is investigated (Mitchell-Olds *et al*, 2007; Symonds *et al*, 2005; Vasemagi and Primmer, 2005). QTL analysis can be augmented by analysis of the natural variation in the trait that exists both between and within populations (e.g. (Heidel *et al*, 2006)). We additionally analyzed variation in glucosinolates and insect feeding within and between four populations from Montana and Idaho.

MATERIALS AND METHODS

Four population analysis

Individuals from four populations from Idaho (ID) and Montana (MT) were grown. The four sites are as follows: Twin Saddle (ID-14), Parker Meadow (ID-70), Ruby Creek (MT-55) and Joseph Creek (MT-56). These populations and individuals have been included in a larger analysis of population structure and linkage disequilibrium (Song *et al*, 2006; Song *et al*, submitted). ID-14 and ID-70 are part of the Northern cluster and MT-55 and MT-56 part of Monida/Western cluster (Song *et al*, 2006; Song *et al*, submitted). We first grew plants under uniform laboratory conditions in the absence of herbivores for one generation in order to remove maternal effects (Agrawal, 2002). We then grew six replicate plants from twenty-four families from each of the four populations for glucosinolate analysis and for feeding experiments with *T. ni* (for a total of 576 plants).

Glucosinolate analysis and insect feeding were done as reported in (Clauss *et al*, 2006). There are several critical differences between the analysis done for these populations and the analysis done in the QTL study. Specifically, in this experiment we added a known amount of an internal sinalbin standard (4-hydroxybenzyl GS) before extraction. This allowed for the calculation of concentrations of all GS compounds in micromole per gram dry weight. Additionally, the concentrations here were calculated from HPLC peak areas using response factors. These response factors are based on those computed for pure desulfo-GS standards at 229 nm (Brown et al., 2003), and recalculated for sinalbin. For our QTL analysis, we did not include an internal standard, and hence values are relative concentrations and not reported in micromols per dry weight.

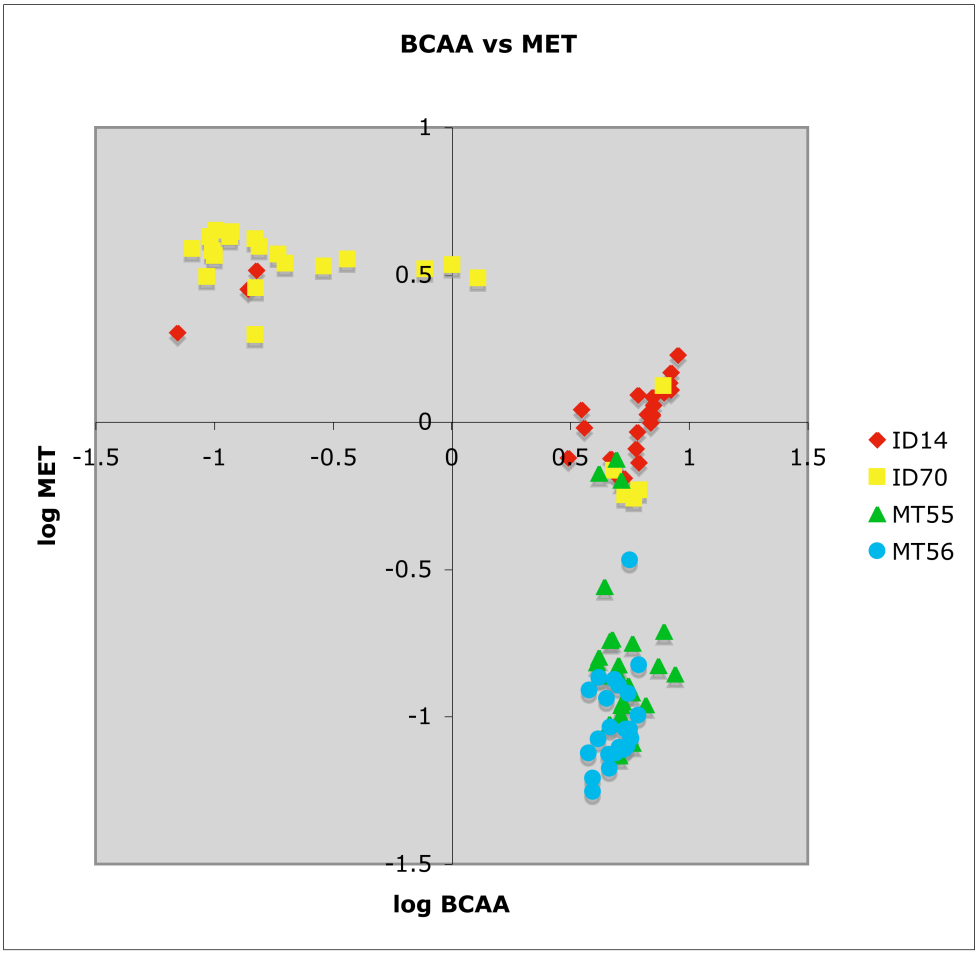
The four population data were analyzed using multivariate genetic analyses (Rausher, 1992), by family-mean ANCOVA Using Systat Software (version 10; Systat Software, Inc., Richmond, CA).

RESULTS AND DISCUSSION

Similar to the variation found within our mapping population, we found major differences between the ratio of methionine and Branched-Chain amino acid derived glucosinolates (Supplemental Figure 1), again, with the major Met glucosinolate being 6MSOH and the major BCAA glucosinolates being 1ME (from Val) and 1MP

(from Ile). Within the two populations from Idaho we found within population variation, whereas the two Montana populations contained mostly BCAA-derived glucosinolates (Supplemental Figure 1). Additionally, there was a significant correlation between the ratio of BCAA/Met glucosinolates with feeding damage by the generalist insect *T. ni*. In multivariate genetic analyses (Rausher, 1992), levels of herbivory by *T. ni* were significantly predicted by population, total glucosinolate concentration, and the proportion of valine- versus methionine-derived glucosinolates (all $P < 0.024$ by family-mean ANCOVA).
Hence, we conclude that the major polymorphism in glucosinolates and insect feeding is not unique to the two parents used to construct or genetic map and perform QTL analysis. It is also segregating both within and between populations. The future cloning of the locus in combination with population genetic analysis, for example from the populations presented here, will allow us to further investigate the potential adaptive value of the trait and evolutionary selective pressures acting on the locus.

Supplemental Figure 1. Plot of the log values for the concentration of methionine (MET) versus branched chain amino acid (BCAA) glucosinolates for each of 24 individuals in each of four populations (for 96 total lines). Glucosinolate concentrations of all GS compounds were calculated as micromole per gram dry weights. The value for each individual was based on the mean from 6 replicate plants. Individuals are from four populations from Idaho (ID) and Montana (MT): Twin Saddle (ID-14), Parker Meadow (ID-70), Ruby Creek (MT-55) and Joseph Creek (MT-56).



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