Phenotypic robustness determines genetic regulation of complex traits

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ABSTRACT

A balance between phenotypic variability and robustness is crucial for populations to adapt to multiple selection pressures. The plasticity of genetic pathways underlies this balance. We investigated this plasticity by studying the regulation of phenotypic mean and variance in a biparental recombinant population of *Saccharomyces cerevisiae* grown in a variety of environments. We found that the growth of this population was well buffered in most environments, such that majority of alleles regulated the mean value of phenotype, and only a subset of these alleles regulated phenotypic variance. This latter class of alleles allowed the other genetic variants to express a range of phenotypic values around a shifted mean. This shift depends on the population and the environment, *i.e.* based on the evolutionary history of a strain, buffering can result in either a superior or an inferior phenotype in an environment but never both. Interestingly, intricate coupling of the genetic network regulating mean phenotype and robustness was observed in a few environments, which highlighted the importance of phenotypic buffering in layout of the genetic architecture. For loci regulating variance, show a higher tendency of genetic interactions, which not only establishes a genetic basis of release of variance, but also emphasizes the importance of mapping robustness in understanding the network topology of complex traits. Our study demonstrates differential robustness as one of the central mechanisms regulating variation in populations and underlines its role in identifying missing heritability in complex phenotypes and diseases.

INTRODUCTION

The effect of genetic variation can either remain dormant or manifest itself phenotypically, a phenomenon referred to as phenotypic capacitance (Masel and Siegal 2009; Félix and Barkoulas 2015; Hayden *et al.* 2015). The process of revelation of phenotypic variability (decanalization) has adaptive potential for populations in novel or stressful environments (Masel 2013). Released mutations, often referred to as cryptic genetic variability (CGV) (Paaby and Rockman 2014) can exhibit high phenotypic variability across multiple environments, which provides adaptive diversity (Chevin *et al.* 2010; Geiler-Samerotte *et al.* 2013). Understanding the processes underlying phenotypic capacitance is important for interpreting evolutionary effects of forces acting on populations, which result in missing heritability and incomplete penetrance of complex traits and disease. Such processes may ultimately govern the origin and development of complex genetic and molecular networks (Siegal and Leu 2014).

Recent advances in systems biology have revealed that interpreting molecular mechanisms of evolution is the key in understanding the highly interconnected genetic map (Wagner *et al.* 2007; Costanzo *et al.* 2010). Certain genes, called hubs, show higher connectivity than others and thus holding the network together (Vidal *et al.* 2011). Deciphering the mechanisms, by which these network hubs modulate the effector genes in an environment- and genetic-background dependent manner to influence the phenotype, is the key to understanding how phenotypic capacitance and variability have created the genotype-phenotype (GP) map.

Despite being proposed over half a century ago (Jamniczky *et al.* 2010), only recently, a few studies have begun to identify possible regulators that buffer this genetic diversity (Félix and Barkoulas 2015). Evidence from various genome-wide deletion, protein-protein and transcriptional network analyses have proposed chromatin regulators as network hubs regulating phenotypic capacitance and variability (Levy and Siegal 2008; Tirosh *et al.* 2010). However, the molecular mechanisms through which these networks mediate the variability in the effector genes and generate diverse phenotypic responses are largely unknown. To date, the mechanistic bases have been identified only in a few cases, *e.g.* Hsp90, a highly conserved protein that regulates the phenotypic variance through altered protein folding (Rutherford and Lindquist 1998; Queitsch *et al.* 2002).

In this study, we have attempted to address the following two questions. How important is regulation of robustness for genetic regulation of the phenotype? And, whether perturbation of this robustness and the subsequent release of CGV are beneficial or detrimental for the population growth?

In order to understand the regulation of phenotypic variability and plasticity, it is necessary to investigate the effect of regulators on diverse mutations, an approach applied so far to only a few genes (Queitsch *et al.* 2012). For a single species, though not achievable through single gene perturbation studies, recombinant populations analyzed in various environments provide an excellent platform to study such effects (Lempe *et al.* 2013). The allelic variants that mediate how phenotypes are regulated have two potential outcomes. First, there is an effect on the 'mean' value of some readout, such as growth in different environments in the case of yeast. Mapping such an effect on population 'mean' values allows the identification of alleles that affect phenotypes independent of the genetic background. In addition, there is a second

87 consequence, *i.e.* an effect on the 'variance' of the readout (Hall *et al.* 2007; Lee *et al.* 2014),
88 which is often ignored in many studies. Alleles that cause such differences in population
89 'variance' have a more subtle effect, whereby genetic diversity is retained in the population
90 (Figure 1A). In our study, we used a synthesis of both these mapping approaches to discern
91 patterns of regulation of variation in a recombinant population. We find that the genetic loci,
92 and their networks, regulating robustness are a subset of the conventionally studied loci,
93 which affect phenotypic mean. However, in certain environments, based on the evolutionary
94 history of the parental strains, these two networks are very closely intertwined as
95 demonstrated by complete loss of buffering in the recombinants. Such environments highlight
96 the importance of mechanisms regulating robustness in the genetic architecture. Based on our
97 analysis, we propose differential robustness of alleles as a major contributor to antagonistic
98 pleiotropy. Finally, we provide evidence for genetic basis of loss of robustness and therefore
99 propose variance mapping as a methodology to uncover the genetic interactions regulating
100 complex traits.
101
102
103 **RESULTS**
104 **High overlap between mean and variance QTL**
105 Using a recombinant population generated from a biparental cross, we carried out linkage
106 mapping to identify genetic loci regulating both phenotypic mean (QTL) and phenotypic
107 robustness (variance QTL or vQTL) of colony size variation across 34 diverse environments
108 ranging from different carbon sources to oxidative and DNA damaging stress (see Methods).
109 To compare the genetic loci identified by QTL and vQTL mapping, the genome was binned
110 into 595 bins (see Methods, Tables S1, S2). The distribution of loci showing a difference in
111 mean only, variance (robustness) only or both, differed across environments (Figure 1B). The
112 majority of all loci identified affected only mean (70%) with 4% affecting only robustness.
113 However, a substantial 27% loci were both QTL + vQTL (Figure 1C). For these loci,
114 pleiotropic hotspots were identified, which regulated mean, robustness or both across
115 multiple environments (Table S2).
116
117 **Environment dependent regulation of phenotypic mean and robustness**
118 While there was no association between number of QTL and vQTL identified across
119 environments, there was a negative correlation (r^2 = -0.4) between the number of QTL and
120 the number of loci which were both QTL + vQTL associated across environments. This

121 indicated that there is a decrease in the number of loci with independent effects as the number
122 of loci regulating variance increase. Based on the overlap between mean and variance loci,
123 we could divide the environments into three categories – (i) No overlap between QTL and
124 vQTL: this indicated that growth was tightly buffered in the recombinants and while the
125 genetic variants affected the mean of the phenotype, robustness was maintained. These
126 environments were enriched in various carbon sources. (ii) Partial overlap between QTL and
127 vQTL: some loci showed difference in robustness but the majority affected only the mean.
128 (iii) Almost complete overlap: in these environments, all loci which affected the phenotypic
129 mean resulted in varying degrees of loss of robustness. In such environments only two states
130 were possible, *i.e.* either the allele contributed to the buffered state or it resulted in loss of
131 robustness. Growth in such environments emphasized the role of robustness in regulating
132 phenotypic variation.
133
134 **Release of phenotypic variance is directional**
135 A high overlap of regulation of mean and robustness indicated that the loci regulating
136 phenotypic variability were a subset of the loci that have been implicated to be associated
137 with a shift in the mean upon release of variation. While pleiotropic hotspots regulated the
138 mean and the variance across multiple environments, no discernible pattern was observed in
139 the directionality of this effect, except when considering each environment independently
140 (Figure 2A). In the majority of environments (26/33), we observed a strong correlation
141 between the effect of the allele on the mean and the variance for the significant loci. One half
142 of the environments (13/26) showed a strong positive correlation ($r^2 > +0.5$) indicating that
143 loss of robustness was, on an average, advantageous, and the other half showed a negative
144 correlation ($r^2 < -0.5$), *i.e.* loss of robustness was detrimental for the population (Figure 2B).
145
146 **Differential regulation of robustness as a major contributor to gene-environment**
147 **interaction**
148 Pleiotropic loci often have antagonistic mean effects across environments (Yadav *et al.*
149 2015). While loci regulating robustness show high environment specificity, we asked if it is
150 possible for a locus to result in robustness in one environment and decanalization in the other
151 by comparing covariance of alleles across environmental pairs (Haber and Dworkin 2015).
152 Forty seven bins were identified that had a significant effect in two or more environments.
153 Eighteen hotspots showed significant difference in the covariance with 10 being significant
154 across multiple pairs of environments (Table S3). Unlike mean effects, high consistency was

155 observed in allelic robustness of a single hotspot across environmental pairs, *i.e.* within a
156 hotspot the same allele showed a low covariance (high buffering or canalized) across multiple
157 environmental pairs and consequently the other allele consistently showed a high covariance
158 (decanalization). This consistency in buffering, when coupled with high environment
159 specificity of directionality (advantageous or deterimental but not both) of release of
160 variance, explains the abundant antagonistic pleiotropy observed in mean effects of these
161 pleiotropic hotspots (Figure S1) (Yadav *et al.* 2015). As suggested by our previous analysis,
162 the environment would determine whether the accumulated mutations would be beneficial or
163 detrimental to the phenotype. In conclusion, this covariance analysis shows that differential
164 regulation of robustness is a major contributor to gene-environment interactions, especially
165 antagonistic pleiotropy.
166
167 **Release of variance is genetic in nature**
168 While most of the polymorphisms in this population were neutral, some showed their direct
169 effects and others were hidden showing their effects only under certain environmental and
170 genetic perturbations. To estimate the extent of genetic basis of loss of robustness, two-QTL
171 interaction mapping was performed by considering the effects of only significant QTL and
172 vQTL loci (see Methods). Amongst the loci showing two-QTL interactions, 10% had only
173 single QTL effects, whereas 80% were either vQTL or both (Table S4) indicating that vQTL
174 were involved in a greater fraction of genetic interactions. These interacting loci showed
175 stronger effects in the decanalized allele than the buffered one (Figure 3B, 3C). In addition to
176 showing that there is a genetic basis regulating the increase in variability, this observation
177 also suggests that altered variance is a good predictor of extent of genetic interactions.
178
179 In our analysis, two kinds of interaction hubs were identified: *within environment interaction*
180 *hubs*, where a locus interacted with multiple loci to regulate phenotypic variation in a given
181 environment; and *across environment interaction hubs*, where a pleiotropic locus interacted
182 with different loci across several environments to regulate phenotypic variation (Figure 4A).
183 Overall, our observations support a modular structure of regulation of phenotypic robustness.
184 In *within environment hubs*, some regulators controlled multiple effector genes in a single
185 environment. In the presence of a decanalized allele, this control is disrupted, resulting in a
186 high variance in the same environment. These hubs were identified in the environments
187 where most loci independently regulated both mean and robustness. On the other hand, in
188 *across environment hubs,* a different set of effectors functionally regulated the phenotype

across distinct environments. This suggests that allelic variations in such hubs will cause changes in variability and control growth across environments.

Network of loci regulate phenotypic buffering

Studies showing high interconnectivity and redundancy in the genetic networks argue for a crosstalk among multiple regulators of phenotypic robustness (Kafri *et al.* 2009; Li *et al.* 2010; Costanzo *et al.* 2010). At a population level, this would translate to maintenance of robustness by a network of loci instead of a single locus. To identify such interactions regulating phenotypic variance, we carried out a two-vQTL interaction mapping using candidate loci (Table S4). To our knowledge, this is the first time that such an analysis has been performed. A large number of two-vQTL interactions were identified, many more than would be expected from proportion of single vQTL analysis, emphasizing the functional relevance of co-regulating variance in population dynamics (Figure 4B). Interestingly, in addition to just the differential regulation of variance, this interaction mapping also identified two-locus pairs which had buffered variance in 3 out of their 4 allelic combinations (Figure 3E, 3F). Indeed, in environments showing high overlap between mean and variance QTL, multiple such pairs were identified (Figure 4C, 4D), indicating that there was a highly connected, redundant multi-locus network that maintained phenotypic robustness. We also observed that it is only under very specific allelic combinations that this network was disrupted to reveal phenotypic variance.

DISCUSSION

Populations balance maintaining a robust phenotype unaffected by genetic perturbation and active accumulation of mutations as potential for evolution. Therefore, it is a reasonable assumption that regulation and mediation of phenotypic variability under different selection pressures plays a significant role in determining the networks governing complex traits (Landry and Rifkin 2010; Geiler-Samerotte *et al.* 2013).

Once a population adapts to a particular environment, stabilizing selection acts to maintain the mean at a specific fitness value. While phenotypically uniform, the population needs to maintain genetic diversity to maintain adaptive potential in the face of a change in selection pressure (Hartman *et al.* 2001; Gibson and Dworkin 2004). This requires the presence of genetic hubs that may sense environmental or genetic perturbations, transducing their

223 responses to the downstream effectors, and therefore, regulate the release of phenotypic
224 capacitance (Figure 5) (Carlborg *et al.* 2006; Le Rouzic and Carlborg 2008; Hayden *et al.*
225 2015). This release of phenotypic diversity can be a result of encountering a novel or a stress
226 environment as well as a result of a change in a regulator (Figure 5) (McGuigan and Sgrò
227 2009).
228
229 Our study showed that high-resolution QTL mapping could be used to distinguish between
230 genetic loci which have a 'mean' effect on the phenotype and those which regulate
231 phenotypic robustness. While various studies have compared loci regulating mean and
232 variance in different organisms in the past they were limited either by sample size or range of
233 phenotypes considered, thus showing varying degrees of overlap between loci regulating
234 mean and variance (Hall *et al.* 2007; Sangster *et al.* 2008; Lee *et al.* 2014). Our study shows
235 empirical evidence that regulation of robustness forms the basis of genetic networks
236 regulating a phenotype. Growth in an environment can be regulated in two ways: either most
237 of the loci affect the mean with only a subset affecting robustness, or almost all loci affect
238 both robustness and the mean. In addition to the nature of the environment, this
239 categorization would depend on the evolutionary history of the parental strains.
240
241 In our study, presence of majority of alleles differentially affected the phenotype mean
242 without perturbing the buffered state, indicates that the network maintaining robustness is
243 extensive and the genetic variation in the population is not sufficient to perturb it.
244 Alternatively, for the environments which parental strains would have encountered during
245 evolution and were similarly adapted to, *viz.*, various carbon sources, diverse parental alleles
246 maintain similar robust state. On the other hand, in a few environments, all alleles that
247 affected mean also affected robustness. This high coupling indicated that robustness was
248 perturbed in the segregating population such that every allele that affected the phenotype also
249 perturbed its robustness. This could be possible if either one of the strains was not exposed to
250 the environment during its evolution or the strains were buffered through incompatible
251 independent mechanisms. In such environments, the network topology and role of robustness
252 in their layout becomes apparent.
253
254 Independent of the kind of environment, strong directionality was observed in the release of
255 cryptic genetic variation. This directionality is likely the frozen-in result of the evolutionary
256 history of the strains or the phenotype (Taute *et al.* 2014). For a stabilized population in a

257 stressful or a novel environment, the population has an evolutionary imperative to release
258 phenotypic variability, resulting in a positive association between the mean and the variance
259 (Le Rouzic and Carlborg 2008; McGuigan and Sgrò 2009). On the other hand, in an adapted
260 environment, release of variability will be detrimental and would be visible as a negative
261 association between the mean and the variance (see Figure 2A). We observed that while
262 genetic loci showed antagonism in their mean effects across environments, they showed high
263 consistency in directionality of their buffering abilities. Environment-dependent effect of
264 release of variance as well as high consistency in buffering indicates that a locus that buffers
265 the phenotypic capacitance will, as a result, have an antagonistic effect on the population
266 mean in an environment-dependent manner. Additionally, this consistency of effect on
267 robustness is in accordance with the known molecular mechanisms. An allele that perturbs
268 protein folding or chromatin silencing (Taylor and Ehrenreich 2015) will show similar
269 molecular behaviour across environments, whether the release will be beneficial or
270 detrimental will be determined by the phenotype. In summary, our study indicates that altered
271 buffering is a potential cause of antagonistic pleiotropy. While we cannot comment on the
272 genes mediating this phenotypic buffering, our study shows that in different environments,
273 different genetic loci or their hubs regulate robustness. This along with identification of
274 environments with high overlap between regulation of mean and robustness support a
275 scenario where multiple genes are involved in buffering the phenotype and may possibly play
276 a crucial role in layout of genetic networks.
277
278 Our variance mapping using biparental recombinant populations adds to the understanding of
279 fundamental questions like missing heritability (Manolio *et al.* 2009; Eichler *et al.* 2010).
280 High population dependence and incomplete penetrance impede the identification of disease-
281 causing alleles (Zuk *et al.* 2012; Mackay 2014). We propose altered phenotypic buffering as a
282 possible causative mechanism behind missing heritability (Queitsch *et al.* 2012). Effect of a
283 disease-causing locus will be neutralized in the presence of an allele that buffers the
284 phenotypic variance, whereas it will be causative in the decanalized allele (Figure 5). While
285 genome-wide association studies suffer from a lack of power to identify such epistatic
286 interactions, analyzing the variance of a population along with the mean can act as a robust
287 refinement to narrow down the possible interactors of disease-causing alleles.
288
289

290 **METHODS**

291 **Dataset**

292 The raw growth data analysed in this study was derived from a study by Bloom *et al*. (2013),
293 in which the experimental procedures are described in detail. The data we used was generated
294 for 1,008 segregants derived from a cross between *S. cerevisiae* strains BY (a laboratory
295 strain) and RM11-1a (a wine isolate, indicated as RM). These segregants were grown in 46
296 different conditions. Of these, we studied 34 conditions (see Table S2, see File S1 for more
297 information).

298

299 **QTL and vQTL mapping**

300 The single environment QTL and two-QTL mapping was carried out as described previously
301 (Bhatia *et al.* 2014).

302

303 To estimate the difference in phenotypic variance between the two genotypic groups, *i.e.* to
304 identify vQTL in each environment, the standard Brown-Forsythe (BF) statistic (Lee *et al.*
305 2014) and the corresponding LOD score were calculated for each genetic marker in each
306 environment (see File S1). The BF test is equivalent to an F-test performed on the deviations
307 of the phenotypic values from their respective genotypic medians (or the means). Hence,
308 under the alternative hypothesis, the phenotypes of the two alleles reveal a difference in the
309 variance. As a result, the corresponding LOD scores indicate markers responsible for genetic
310 canalization defined as variance-QTL (vQTL).

311

312 At a particular marker, let z_{ij} be the absolute deviation of segregant *i*'s phenotypic value y_{ij}
313 from its genotypic mean $\tilde{y}_j$ where j can take two values ($j=1$: BY allele and $j=2$: RM
314 allele).

315 $$z_{ij} = \left| y_{ij} - \tilde{y}_j \right|$$

316

317 Then BF statistic for that marker can be computed as follows:

318 $$F = \frac{(N-p)}{(p-1)} \frac{\sum_{j=1}^{p} n_j (\tilde{z}_{.j} - \tilde{z}_{..})^2}{\sum_{j=1}^{p} \sum_{i=1}^{n_j} (z_{ij} - \tilde{z}_{.j})^2}$$

319

320 Here, N is the total number of segregants, n_1 and n_2 are the number of segregants having
321 the BY and RM allele respectively ($p = 2$). In order to estimate the effects of vQTL in the
322 same order as in QTL, LOD scores were computed as described previously (Broman and Sen
323 2009).
324
325 To establish the statistical significance of the putative QTL and vQTL, P-values were
326 computed using a genome-wide permutation test of 1,000 permutations, where the null
327 distribution consisted of the highest genome-wide LOD score obtained from each
328 permutation. A LOD cut off of 3.0 and a P-value cut off of 0.01 was considered.
329
330 To estimate pleiotropy, we divided the genome into 20kb non-overlapping bins (Table S1).
331 Bins containing two or more QTL or vQTL significant (P-value < 0.01) in different
332 environments were considered as pleiotropic bins. The first markers of each of these
333 pleiotropic bins, used as representative of the bins were collated to represent the set of
334 pleiotropic markers (Table S1, S2).
335
336 Apart from the standard two-QTL mapping described previously (Bhatia *et al.* 2014), we
337 mapped variance-controlled interactions, *i.e.* a two-vQTL interaction, which occurs when the
338 phenotypic variance at one locus depends on the genotype at another locus. To increase
339 power to identify two-QTL and two-vQTL interactions, for environment specific, targeted
340 multi-QTL mapping, genetic loci significant in either QTL or vQTL or both mappings were
341 collated for each environment (Table S2). Additionally the size of the bin was increased from
342 20kb to 50kb for the same. This collated set of environment-specific loci was tested for both,
343 two-QTL and two-vQTL interactions in their respective environments. A total of 47 two-
344 QTL interactions each significant (P-value < 0.001) in at least one of the 34 environments
345 were obtained. The P-values were computed using a permutation test of 10,000 permutations
346 with the phenotype data shuffled relative to the genotype data.
347
348 **Covariance across environmental pairs**
349 To assess the differential covariance of a locus across multiple environments, we considered
350 the collated set of pleiotropic markers for our study (Table S3). To quantify the differential
351 covariance across a pair of environments, a Deming regression was calculated between the
352 phenotype values of the chosen pair of environments for each allele, using R package 'mcr'.

353 Deming regression, which minimizes errors in multiple dimensions simultaneously, served as
354 a suitable measurement error model for assessing buffering across two or more environments.
355 For every possible environment pair, a t-test was performed between the deviations of the
356 phenotypic values from the Deming fit of the BY and RM allele (P-value < 0.05).
357
358
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364
365
366 **AUTHOR CONTRIBUTIONS**
367 Conceived and designed analysis: AY HS. Analysed data: KD AY HS. Wrote the paper: AY
368 HS.
369
370
371 **FIGURE LEGENDS**
372 **Figure 1: QTL and vQTL**
373 (A) Schematic showing three categories of QTL mapped. QTL has significantly different
374 allelic mean but non-significant different allelic variance; vQTL has non-significant mean
375 difference but significantly different variance; QTL+vQTL has both significant different
376 allelic mean and variance.
377 (B) Distribution of QTL mapped in each environment as QTL (red), vQTL (blue) and
378 QTL+vQTL (black) in the segregating population. The y-axis is proportion of QTL in each
379 category (see Results). The x-axis is arranged by increasing proportion of QTL and the left
380 group has the highest proportion of QTL + vQTL. See Table S2 for details.
381 (C) Total distribution of QTL mapped across 34 environments as QTL (red), vQTL (blue)
382 and QTL+vQTL (black) in the segregating population.
383
384 **Figure 2: Directionality of alleles in QTL+vQTL category**
385 (A) Representative frequency distributions of three QTL markers showing directionality of
386 variance release. Blue distribution is of segregants with BY allele and red is for RM

387 segregants. 4NQO [chrXII (207,340)] marker shows a negative release of variance of BY
388 allele (with RM mean > BY mean); Lactose [chrXIV (376,315)] marker shows equal variance
389 of the two alleles; $MgSO_4$ [chrVII (187,538)] marker shows a positive release of variance of
390 BY allele (with RM mean < BY mean). The QTL are indicated as chromosome number
391 followed by marker position in bp within brackets. Color bar on the top of each distribution
392 represents the three classes of correlations between mean and variance.
393 (B) Correlation between mean and variance of all QTL in each environment. Correlations less
394 than -0.5 (red) represent environments that have a negative release of variance of BY allele.
395 Correlations more than +0.5 (yellow) represent environments that have a positive release of
396 variance of BY allele. Environments with no directional release of variance (correlations
397 between ±0.5) are represented as blue. The y-axis is correlation between mean and variance;
398 dashed lines show ±0.5 correlation value.
399
400 **Figure 3: Representative covariance and two-QTL interactions**
401 (A) Covariance of normalized growth phenotype of BY (blue) and RM (red) segregants for
402 chrXIV (368,185) marker in 4-HBA and Galactose. Mean and variance of each allele in each
403 environment is indicated in the box.
404 (B) Two-QTL interaction between chrXIV (368,185)-chrXIII (46,758) in 4-HBA.
405 (C) Two-QTL interaction between chrXIV (368,165)-chrXV (555,452) in Galactose.
406 (D) Covariance of normalized growth phenotype of BY (blue) and RM (red) segregants for
407 chrXII (649,260) marker in $MgSO_4$ and Cu (copper). Mean and variance of each allele in
408 each environment is indicated in the box.
409 (E) Two-QTL interaction between chrXII (649,260)-chrVII (187,538) in $MgSO_4$.
410 (F) Two-QTL interaction between chrXII (649,260)-chrVIII (208,560) in Cu.
411 The QTL is indicated as a chromosome number followed by the marker position in bp within
412 brackets (a convention followed in all QTL plots). For (A, D), the axes are normalized
413 growth of segregants in the two environments indicated. For (B, C, E, F), the x-axis is
414 normalized growth of segregants in the environment and the y-axis of number of segregants.
415 Dash lines in segregant distributions indicate the means of the distributions. The biallelic
416 marker segregant distributions (in the QTL marker order written above the plots) are
417 indicated as BB (light blue), BR (light brown), RB (dark green) and RR (purple). Inset plots
418 show the average distributions of the first marker (BY (blue) and RM (red) alleles). See Table
419 S3, S4 for details.
420

421 **Figure 4: Two-QTL and two-vQTL interactions**

422 (A) Two-QTL and two-vQTL interactions between various markers shown as connected
423 links. The chrII (245,879) marker (red) has multiple two-QTL interactions (deep red) for
424 growth in same environment (Congo red). The chrXIV (466,590) marker (green) has 6
425 environment-specific two-QTL interactions (4NQO= dark green, Formamide= deep purple,
426 IAA= light blue, Lithium chloride= deep orange, Trehalose= deep yellow, Xylose= orange).
427 Other two-QTL interactions are indicated as light grey links.

428 (B) All two-QTL (red), two-vQTL (blue) and two-QTL+vQTL (black) interactions across all
429 environments.

430 The figures were made using Circos (Krzywinski *et al.* 2009). See Table S4 for data.

431 (C) Scatter plots showing examples of two-QTL interactions of four markers [chrIII
432 (191,928), chrIV (997,621), chrVIII (101,016), chrXIV (466,105)] in Indoleacetic acid. The
433 biallelic marker segregant distributions (in the QTL marker order written above the plots) are
434 indicated as BB (red), BR (yellow), RB (green) and RR (blue) on x-axis. Mean and variance
435 of each allelic pair is indicated in the box with allelic pair with most variance indicated in red.
436 The y-axis is normalized growth phenotype.

437 (D) Schematic representation of network of four loci (indicated in (C) above) maintaining
438 phenotypic robustness in Indoleacetic acid.

439

440 **Figure 5: A schematic showing environment and genetic background dependent loss of**
441 **buffering**

442 A regulator (red node) of robustness buffers genetic variability (blue nodes), which results in
443 a robust population phenotype. This buffering is lost in either a differential allele (green
444 node) of the regulator or a novel environment (red distribution), thus releasing cryptic genetic
445 variability (yellow node), which in favourable cases results in better adaptation in the novel
446 environment, or in unfavourable cases manifests as a detrimental phenotype or a disease.

447

448

449 **SUPPORTING INFORMATION**

450 **File S1**: Scripts and datasets for QTL, vQTL, and two-QTL, two-vQTL interaction mapping.

451

452 **Table S1**: Classification of chromosomal markers into bins.

453 **Table S2**: List of markers significant for QTL and vQTL mapping. F and BF statistics along
454 with their P-values along with the categorization as only vQTL, only QTL and both.

455 **Table S3**: Covariance analysis of selected bins. T-test of Deming regression between the two
456 alleles across environmental pairs along with its mean and variance for each allele, mean and
457 variance of each allele independently in both environments.
458 **Table S4**: List of markers significant for two-QTL and two-vQTL mapping.
459
460 **Figure S1**: Covariance of normalized growth phenotype of BY (red) and RM (blue) allele
461 chrXIII (45,801) marker in Paraquat and Copper. Mean and variance of each allele in each
462 environment is indicated in the box.
463
464
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570

Figure 1

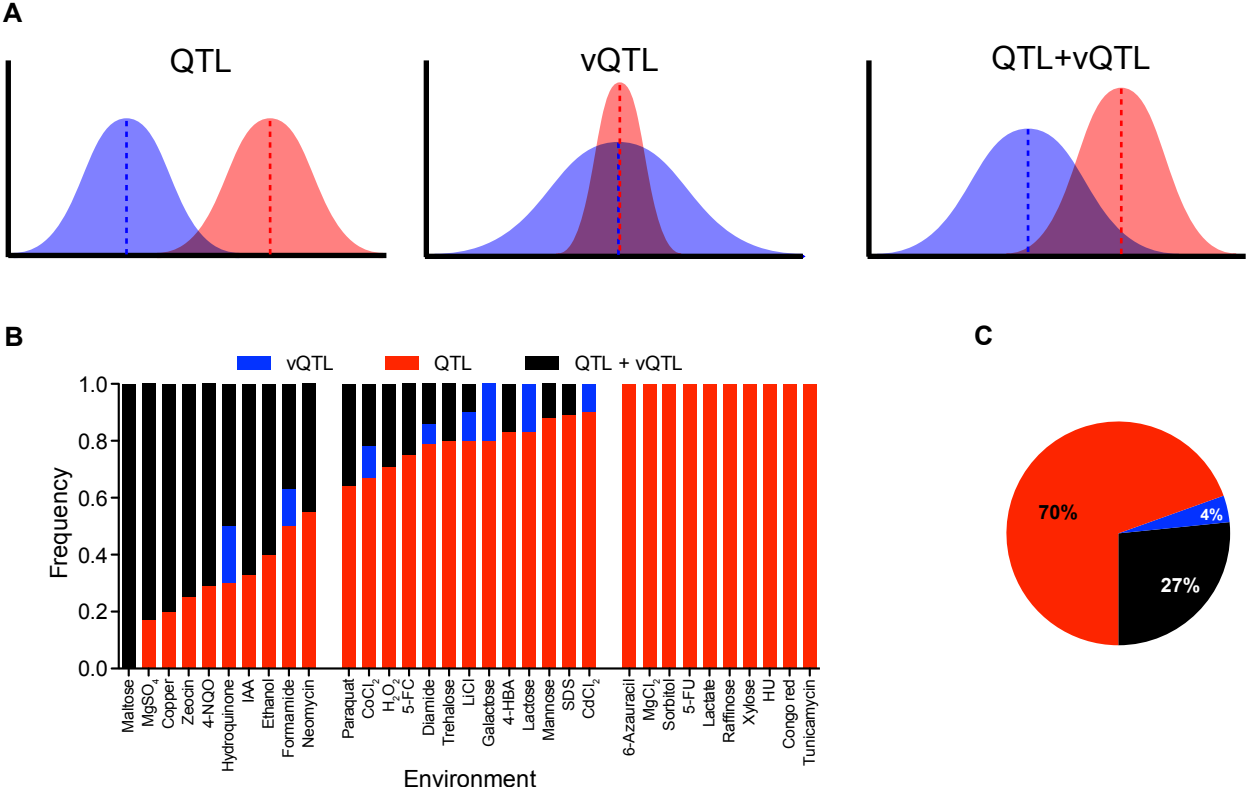

A
QTL
vQTL
QTL+vQTL
B
vQTL
QTL
QTL + vQTL
Frequency
1.0
0.8
0.6
0.4
0.2
0.0
Maltose
MgSO4
Copper
Zeocin
4-NQO
Hydroquinone
IAA
Ethanol
Formamide
Neomycin
Paraquat
CoCl2
H2O2
5-FC
Diamide
Trehalose
LiCl
Galactose
4-HBA
Lactose
Mannose
SDS
CdCl2
6-Azauracil
MgCl2
Sorbitol
5-FU
Lactate
Raffinose
Xylose
HU
Congo red
Tunicamycin
Environment
C
70%
4%
27%

Figure 2

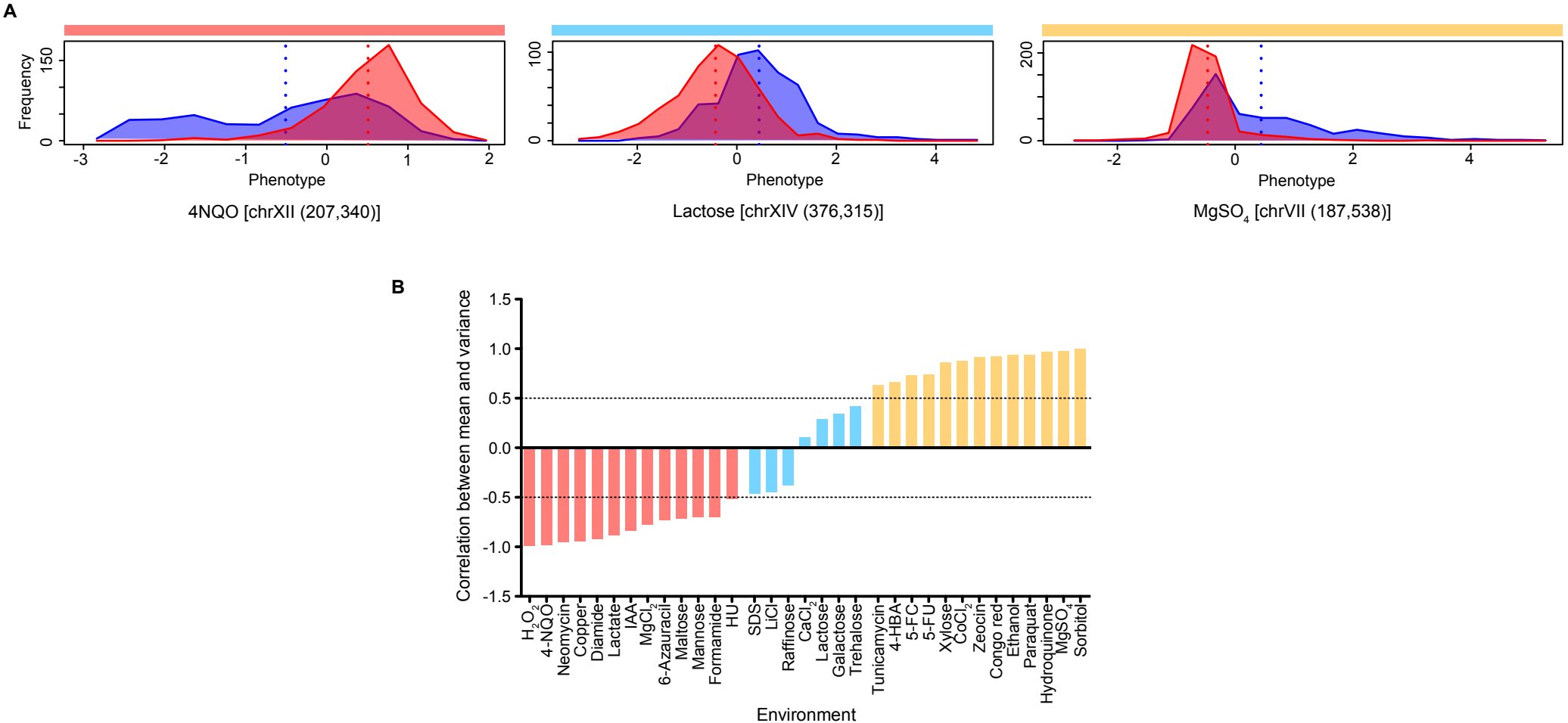

A
Frequency
Phenotype
4NQO [chrXII (207,340)]
Lactose [chrXIV (376,315)]
MgSO4 [chrVII (187,538)]
B
Correlation between mean and variance
Environment
H2O2
4-NQO
Neomycin
Copper
Diamide
Lactate
IAA
MgCl2
6-Azauracil
Maltose
Mannose
Formamide
HU
SDS
LiCl
Raffinose
CaCl2
Lactose
Galactose
Trehalose
Tunicamycin
4-HBA
5-FC
5-FU
Xylose
CoCl2
Zeocin
Congo red
Ethanol
Paraquat
Hydroquinone
MgSO4
Sorbitol

Figure 3

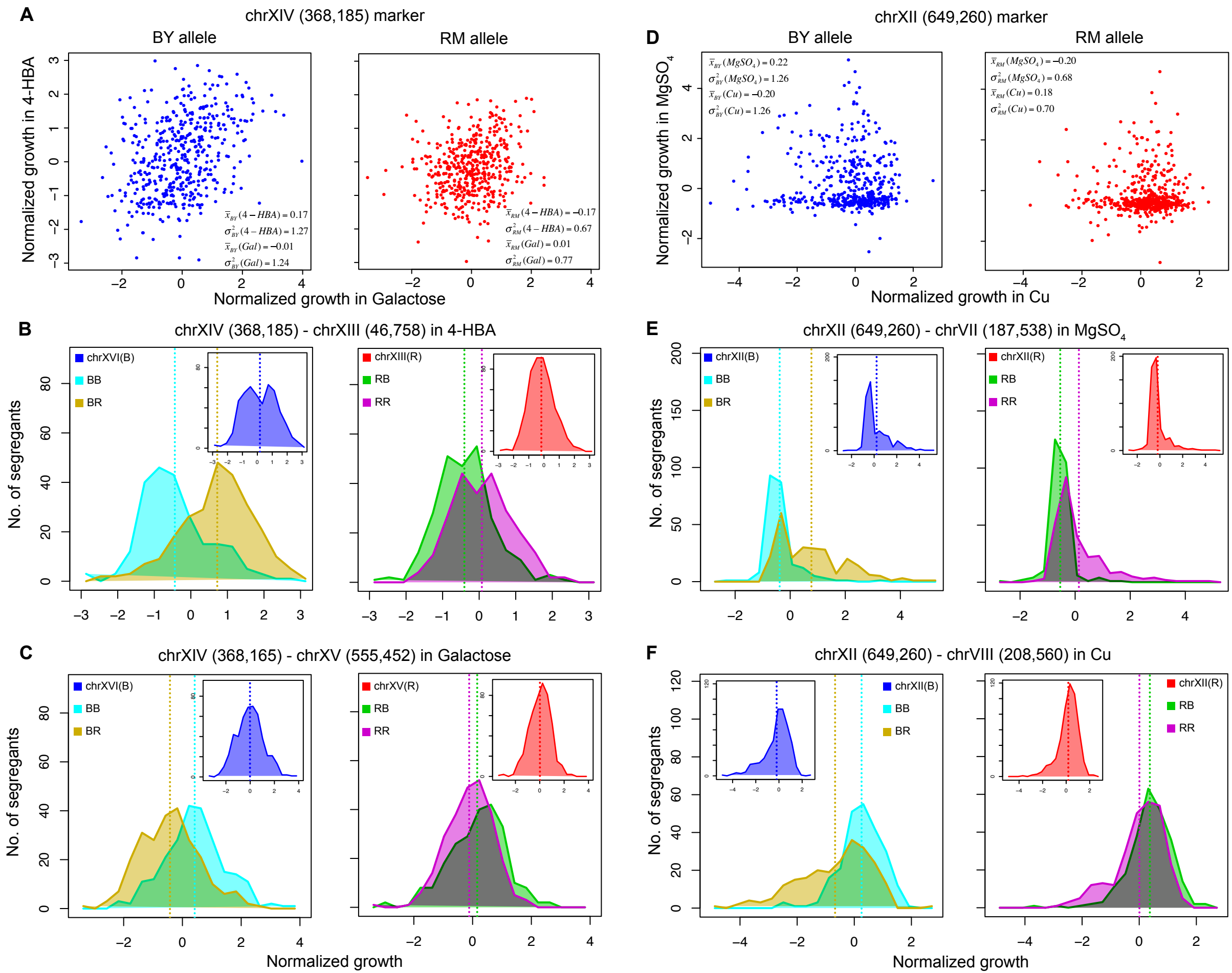

A
chrXIV (368,185) marker
BY allele
RM allele
Normalized growth in 4-HBA
Normalized growth in Galactose
$\bar{x}_{BY}(4-HBA) = 0.17$
$\sigma^2_{BY}(4-HBA) = 1.27$
$\bar{x}_{BY}(Gal) = -0.01$
$\sigma^2_{BY}(Gal) = 1.24$
$\bar{x}_{RM}(4-HBA) = -0.17$
$\sigma^2_{RM}(4-HBA) = 0.67$
$\bar{x}_{RM}(Gal) = 0.01$
$\sigma^2_{RM}(Gal) = 0.77$
D
chrXII (649,260) marker
BY allele
RM allele
Normalized growth in $MgSO_4$
Normalized growth in Cu
$\bar{x}_{BY}(MgSO_4) = 0.22$
$\sigma^2_{BY}(MgSO_4) = 1.26$
$\bar{x}_{BY}(Cu) = -0.20$
$\sigma^2_{BY}(Cu) = 1.26$
$\bar{x}_{RM}(MgSO_4) = -0.20$
$\sigma^2_{RM}(MgSO_4) = 0.68$
$\bar{x}_{RM}(Cu) = 0.18$
$\sigma^2_{RM}(Cu) = 0.70$
B
chrXIV (368,185) - chrXIII (46,758) in 4-HBA
chrXVI(B)
BB
BR
chrXIII(R)
RB
RR
No. of segregants
E
chrXII (649,260) - chrVII (187,538) in $MgSO_4$
chrXII(B)
BB
BR
chrXII(R)
RB
RR
No. of segregants
C
chrXIV (368,165) - chrXV (555,452) in Galactose
chrXVI(B)
BB
BR
chrXV(R)
RB
RR
No. of segregants
Normalized growth
F
chrXII (649,260) - chrVIII (208,560) in Cu
chrXII(B)
BB
BR
chrXII(R)
RB
RR
No. of segregants
Normalized growth

Figure 4

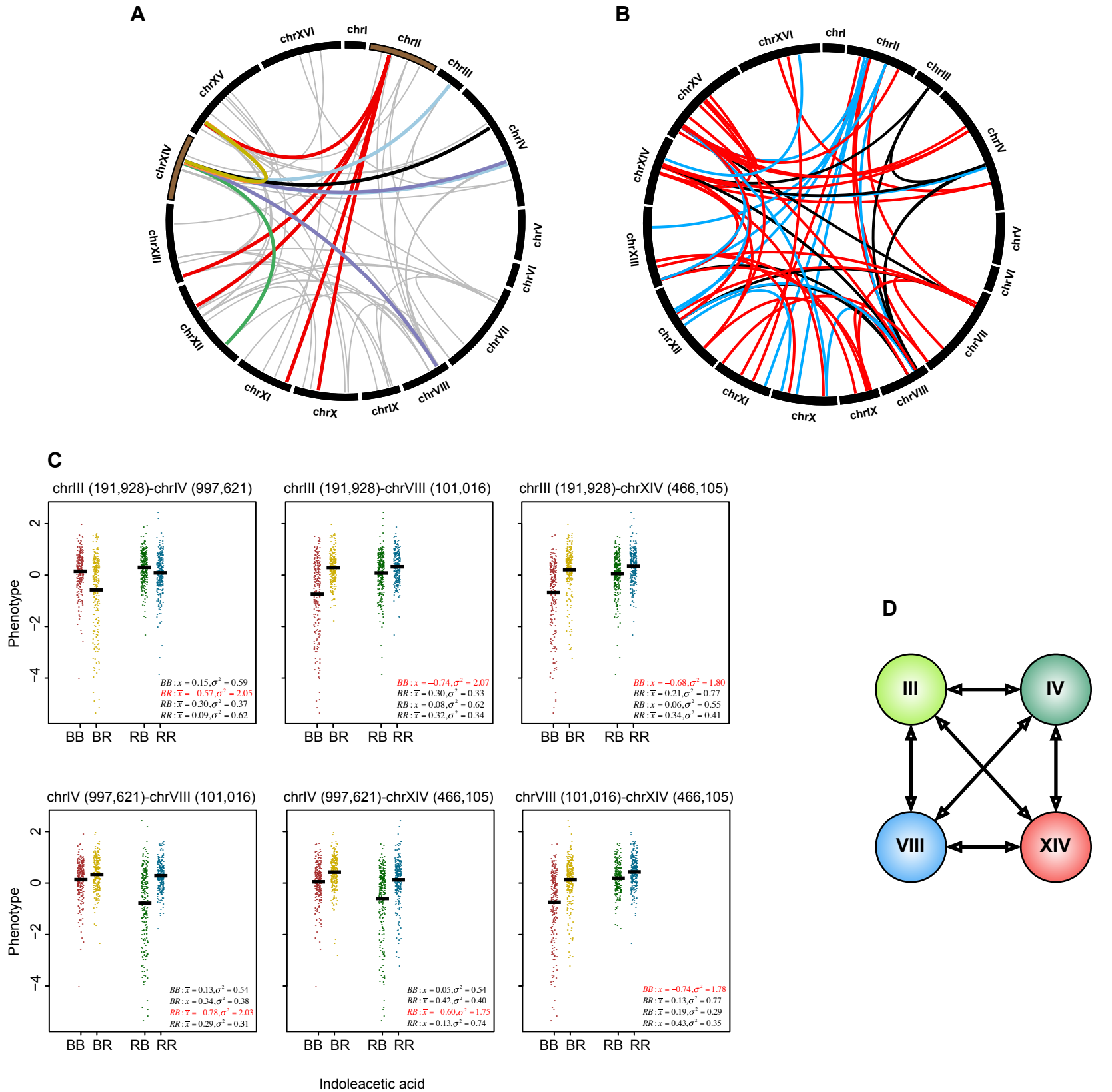

A
B
chrI
chrII
chrIII
chrIV
chrV
chrVI
chrVII
chrVIII
chrIX
chrX
chrXI
chrXII
chrXIII
chrXIV
chrXV
chrXVI
C
chrIII (191,928)-chrIV (997,621)
chrIII (191,928)-chrVIII (101,016)
chrIII (191,928)-chrXIV (466,105)
chrIV (997,621)-chrVIII (101,016)
chrIV (997,621)-chrXIV (466,105)
chrVIII (101,016)-chrXIV (466,105)
Phenotype
BB BR RB RR
Indoleacetic acid
D
III
IV
VIII
XIV

Figure 5

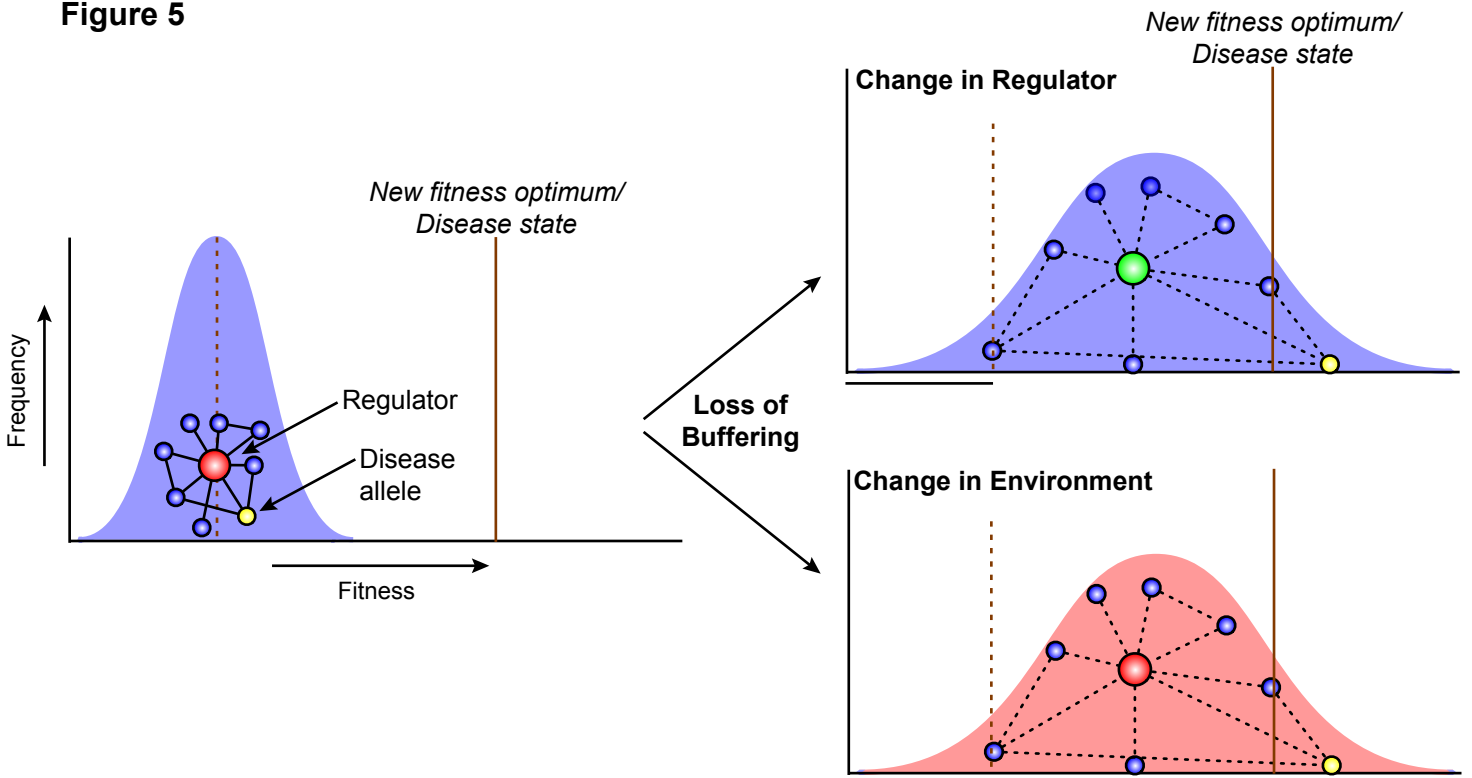
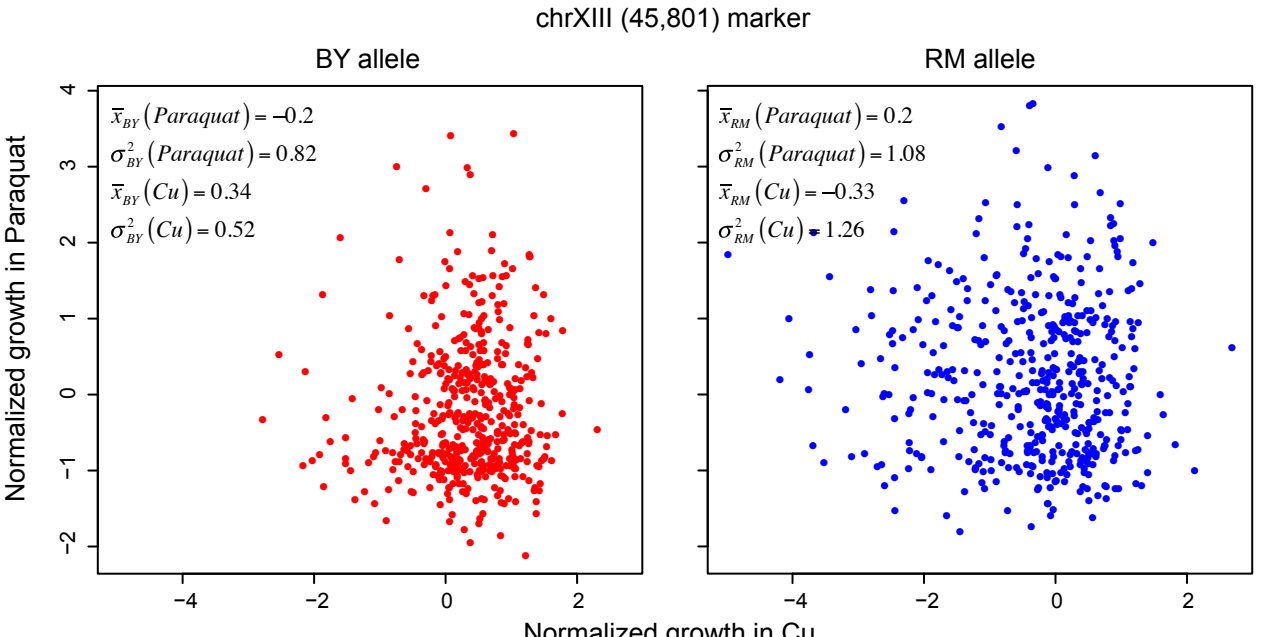

New fitness optimum/
Disease state
Frequency
Regulator
Disease
allele
Fitness
Loss of
Buffering
Change in Regulator
New fitness optimum/
Disease state
Change in Environment

Figure S1


chrXIII (45,801) marker
BY allele
RM allele
$\bar{x}_{BY}(Paraquat) = -0.2$
$\sigma^2_{BY}(Paraquat) = 0.82$
$\bar{x}_{BY}(Cu) = 0.34$
$\sigma^2_{BY}(Cu) = 0.52$
$\bar{x}_{RM}(Paraquat) = 0.2$
$\sigma^2_{RM}(Paraquat) = 1.08$
$\bar{x}_{RM}(Cu) = -0.33$
$\sigma^2_{RM}(Cu) = 1.26$
Normalized growth in Paraquat
Normalized growth in Cu
4
3
2
1
0
−1
−2
−4
−2
0
2