Unraveling the Genetic Architecture of Cryptic Genetic Variation

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Short title

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ABSTRACT

Cryptic genetic variation (CGV), hidden under most conditions, is the repressed genetic potential that can facilitate adaptation and evolution. The conditional manifestation of CGV has been claimed to explain the background dependence of causal loci as well as missing heritability. However, despite being proposed over 60 years ago, the genetic architecture and regulation of CGV and its contribution towards regulation of complex traits remains unclear. Using linkage mapping of mean and variance effects, we have identified loci that regulate phenotypic manifestation of standing genetic variation in a previously published dataset of biparental *Saccharomyces cerevisiae* population grown in 34 diverse environments. Based on our results we propose the existence of a gradient of buffering states for a population determined by the environment. Most environments show a tight buffering with additive, independent causal loci with little epistasis. However, as this buffering is disrupted, the underlying highly interconnected environment-specific genetic interactome is revealed such that each causal locus is a part of this network. Interspersed within these networks are generalist capacitors that regulate CGV across multiple environments, with one allele behaving as a capacitor and the other as a potentiator. Our study demonstrates the connecting link between architecture of hidden and visible genetic variation and uncovers the genetic networks which potentially underlie all complex traits. Our study establishes CGV as a significant contributor to phenotypic variation, and provides evidence for a predictable pattern underlying gene-gene and gene-environment interactions that can explain background dependence and missing heritability in complex traits and diseases.

SUMMARY

The phenotypic effects of cryptic genetic variation (CGV) are mostly hidden and manifested only under certain rare conditions and have the potential to facilitate adaptation. However, little is understood about its genetic regulation. We performed variance QTL mapping to understand the regulation of phenotypic manifestation of standing genetic variation in a biparental yeast population. We propose a model describing the connecting link between visible variation and CGV. We identify generalist capacitors and environment-specific networks that potentially underlie all phenotypes. This fresh approach of mapping causal loci can solve the long-standing mystery of missing heritability in complex traits and diseases.

INTRODUCTION

Cryptic genetic variation (CGV) is a heritable variation that is phenotypically inactive or hidden under most conditions, but manifests phenotypically only under certain, often rare, genetic and environmental perturbations (Gibson and Dworkin 2004). In simpler terms, it is bottled up genetic potential (Gibson and Reed 2008). While described in some studies as a separate and an inexplicable class of genetic variation, it essentially refers to gene-environment interactions, *i.e.*, genetic variants show their phenotypic effects only in certain environments or conditional neutrality, and gene-gene interactions, *i.e.* variants show their effects in the certain genetic backgrounds or epistasis. While the idea of CGV was proposed almost 60 years ago (Waddington 1956), for decades, both population genetic studies as well as evolutionary hypotheses have focused on the additive effects of mutations. Low resolution of mapping techniques curtailed the identification of large-scale gene-environment and gene-gene interactions. With recent advances in sequencing technologies and mapping resolution (Liti and Louis 2012; Siegal 2013), the prevalence of CGV in quantitative traits is being recognized. Environment specificity and epistasis identified in multiple studies to understand the architecture of complex traits in model systems, plant and animal breeding or to study environment and background dependence of human disease alleles, have established CGV as an extensive contributor to the genetic landscape of quantitative traits (Gibson 2009; Queitsch *et al.* 2012).

Introgression lines in classical systems like *Drosophila* and plants show that an allele can show a range of effects depending on the genetic backgrounds an allele is functional in (Gibson *et al.* 1999). Comparative studies in genetically accessible yeast have shown that the majority of loci that affect growth, an important fitness phenotype, have environment or genetic background dependent effects (Cubillos *et al.* 2011; Matsui *et al.* 2015). The importance of CGV in human traits is exemplified by increasing prevalence of diseases like type 2 diabetes. A highly heritable disease, type 2 diabetes, shows both population specificity and lifestyle dependence. Its high heritability is due to exposure of CGV by the modern culture (Gibson and Reed 2008; Gibson 2009).

While prevalence and impact of CGV is being demonstrated by increasing number of recent studies, its regulation is still unclear. Whether CGV is randomly distributed without any discernible patterns or is it controlled by specific genes or genetic networks is one of the key debates in the field, understanding of which will provide an ability to study the CGV

82 landscape and predict its effects on diverse phenotypes (Hermisson and Wagner 2004). To
83 address this question, it is important to understand how does CGV accumulate. Multiple
84 evolutionary hypotheses and molecular explanations have been provided to explain the
85 existence of CGV. Kimura's neutral theory of evolution states that most mutations that
86 accumulate in a population are phenotypically neutral (Kimura 1977; Kitano 2004).
87 Waddington (1956) proposed an explanation for this neutrality by stating that if the mutations
88 are disadvantageous to the optimum phenotype their effects will be hidden or dampened
89 resulting in an invariant population phenotype, a process known as *canalization*. These
90 dampening mechanisms could be altered upon genetic or environmental perturbations, thus
91 revealing heritable variation which could facilitate the process of adaptation (Le Rouzic and
92 Carlborg 2008; McGuigan and Sgrò 2009). The process of canalization, proposed by
93 Waddington, invoked two types of genetic buffering mechanisms (Hartman *et al.* 2001;
94 Burga *et al.* 2012) that contain the effects of CGV in stabilized, well adapted populations –
95 generic buffering systems called *capacitors* (Rutherford and Lindquist 1998) and specific
96 buffering systems consisting of *genetic networks* (Carlson and Doyle 2002). Capacitors are
97 the genes that have the ability to suppress and hence store large amount of genetic variation.
98 Upon specific environmental cues or genetic perturbations, these capacitors would, therefore,
99 yield high CGV, that would affect all fitness phenotypes. In an adapting population, the
100 released CGV can either be advantageous or detrimental (Orr and Betancourt 2001).
101 However, in an adapted population, release of CGV would be detrimental since the
102 population already exists at its phenotypic optimum (Barton and Turelli 1989; Hoffmann and
103 Merilä 1999). As a result, such capacitors are proposed to have pleiotropic effects.
104 Theoretically, such evolutionary capacitors should demonstrate storage of CGV as well as
105 promote an increased ability to accumulate mutations or *mutational robustness* (Draghi *et al.*
106 2010; Fares 2015). The second type of genetic buffering could arise when stabilizing
107 selection for a particular phenotype results in evolution of specific genetic networks, which
108 buffer genetic variation in that particular phenotype (Masel and Siegal 2009; Paaby and
109 Rockman 2014). These systems have different consequences on evolution as well as on
110 molecular regulation of various traits.

111 Various studies have provided both direct and indirect molecular evidence for both these
112 buffering mechanisms. Waddington's initial studies in *Drosophila* lead to detailed
113 investigation in the same system, which resulted in identification of genes with the ability to
114 store CGV – *Ultrabithorax* (Gibson *et al.* 1999), EGFR (Dworkin *et al.* 2003) and, the most

115 commonly studied gene, *HSP90* (Rutherford and Lindquist 1998). These genes result in
116 revelation of cryptic variants with diverse effects on the phenotype upon their perturbation.
117 Studies of Hsp90, across species, have demonstrated its generalized effect in regulating CGV
118 in diverse phenotypes in yeast, worms, flies, plants and fish (Jarosz *et al.* 2010). Other more
119 recently identified examples include *HZT1* (Richardson *et al.* 2013) and *IRA2* (Taylor and
120 Ehrenreich 2015) in yeast. Alternately, molecular compensation through duplicate genes,
121 redundant pathways (Wu and Lai 2015) and high genetic crosstalk have been proposed as
122 specialized networks that contain CGV. A study showed that secondary enhancers of
123 *Shavenbaby* are required to regulate variation in trichome patterning upon extreme stress in
124 *Drosophila*, thus demonstrating a phenotype specific network (Frankel *et al.* 2011).
125 Indirectly, high-resolution mapping of the cellular interactome (Boone *et al.* 2007; Li *et al.*
126 2010; Laufer *et al.* 2013) have revealed independent networks that are employed in specific
127 traits and diseases, called modules (Vidal *et al.* 2011). This modularity may indicate
128 phenotype specific genetic networks, which would have evolved in response to stabilizing
129 selection to contain CGV in a specialized manner.

130 While CGV has been proposed to accumulate in a stabilized population and revealed in an
131 adapting population, the accumulation of CGV and genetic buffering are not necessarily
132 synonymous processes (Gibson and Reed 2008; Paaby and Rockman 2014; Siegal and Leu
133 2014). CGV can be a result of conditional effects of alleles that are maintained by mutation-
134 selection balance and genetic drift across populations. These variants may have conditional
135 effects on specific biochemical pathways, which differ in different populations (Chandler *et*
136 *al.* 2013; Chari and Dworkin 2013; Chandler *et al.* 2014). Thus a large amount of such CGV
137 can hence exist with no perturbations in the underlying robustness of the system, as
138 conditional epistasis (Gjuvsland *et al.* 2007). This idea is supported by a lack of association
139 between ability to release CGV and accumulate mutations (*mutational robustness*). While
140 Hsp90 has been shown to facilitate release of hidden genetic variants (Jarosz *et al.* 2010;
141 Rohner *et al.* 2013) that may eventually aid in adaptation (Orr and Betancourt 2001), it has
142 not been demonstrated to support accumulation of novel mutations. The only study that
143 directly tested the two phenomena, release of CGV and mutation robustness, compared the
144 abilities of Htz1, the gene encoding histone H2A.Z (Richardson *et al.* 2013). While Htz1
145 showed high epistasis, CGV was released both in presence and absence of the gene, with no
146 effect on accumulation of novel mutations. Additionally, even Hsp90 has been shown to act
147 as both a capacitor (contain CGV) and as a potentiator (release CGV) (Cowen and Lindquist

148 2005). Genetic mapping both in functional and repressed Hsp90 states resulted in different,
149 but equal number of genetic loci regulating yeast growth (Jarosz and Lindquist 2010). While
150 such studies do not question the relevance of studying CGV to understand the genetic
151 landscape of complex traits, they propose that loss of robustness is just one of the
152 mechanisms that results in release of CGV (Gibson and Reed 2008; Paaby and Rockman
153 2014; Siegal and Leu 2014). However, the extent of overlap between the two phenomena is
154 not clear. Additionally, the co-existence of generalist capacitors and specialized networks is
155 not well understood, owing to exclusivity of the studies investigating these two phenomena.
156 Are capacitors a part of specialized networks? Or is their existence independent of the
157 networks active in different phenotypes? Several network based studies further challenge the
158 evolution of such generalized capacitors, with an incentive to maintain phenotypic
159 robustness, by showing that biological networks have evolved to maintain robustness such
160 that multiple genes can behave as capacitors (Bergman and Siegal 2003). High epistasis and
161 environment dependence of this epistasis further backs the case for CGV being a part of all
162 the biological processes (Hermisson and Wagner 2004).

163 In this study, we have studied the phenotypic manifestation of standing genetic variation to
164 address two key questions in the field. How common is regulation and release of CGV? Is
165 CGV regulated by a few independent pleiotropic capacitors that function across environments
166 or is it a property of certain environment-specific network modules? How do these
167 generalized and specific forms of genetic buffering overlap?

168 We have used linkage mapping to identify loci that regulate CGV in a biparental yeast
169 population grown in diverse environments. These genetically diverse parental strains (BY and
170 RM11) have accumulated large number of polymorphisms over the course of their
171 evolutionary trajectories (Liti *et al.* 2009; Bloom *et al.* 2013). Linkage mapping in such
172 populations allows comparison of the effect of the two divergent alleles of a locus on
173 thousands of genetic variants. While conventional quantitative trait locus (QTL) mapping
174 identifies genetic loci, alleles of which have different effects on the population mean, their
175 effects on population variance are often ignored (Rönnegård and Valdar 2012). While
176 differential mean of the two alleles would demonstrate the effect of the two alleles on the
177 average phenotype, differential variance would convey that while the population is invariant
178 or canalized in the presence of one allele, the other allele allows the phenotypic manifestation
179 of diverse variants that results in a high variance. Therefore, differential variance represents
180 differential ability to regulate CGV (Lempe *et al.* 2013). The term to describe these variance-

181 regulating loci, variance QTL (vQTL) was first introduced by Rönnegård and Valdar (2011).
182 While most of these loci tend to be small effect when compared with conventional QTL
183 (Shen *et al.* 2012), some large effect vQTL such as *MOT1* (Forsberg *et al.* 2015) and *nFT*
184 (Lee *et al.* 2014) have been identified in plants. In addition, vQTL mapping has been
185 proposed to be a predictor of prevalence of gene-gene interactions, further supporting
186 differential regulation of CGV by these loci (Paré *et al.* 2010; Rönnegård and Valdar 2012).

187 This differential variance between allelic effects is different from micro-canalization. Micro-
188 canalization is another form of robustness that refers to phenotypic variation within two
189 genetically identical individuals, or clonal variation (Bergman and Siegal 2003; Levy and
190 Siegal 2008). Here, we have specifically investigated loci, which regulate suppression and
191 revelation of heritable genetic variation.

192 We used variance QTL (vQTL) mapping to identify loci, which show the ability to regulate
193 CGV across diverse environments. We further used covariance analysis to study the
194 pleiotropy of their variance regulation and identified the revealed CGV through QTL-QTL
195 and vQTL-vQTL interaction analyses. We identify a gradient of types of environments that
196 determines the regulation of CGV within them. Most environments are tightly regulated such
197 that the majority of the loci show additive effect without vQTL and epistasis interactions.
198 Only in a few rare environments, this robust state is perturbed such that almost all loci
199 regulate both mean and variance. Such environments reveal high number of epistatic
200 interactions that regulate yeast colony size. It is in these environments, where we compared
201 effect and abundance of generalist capacitors and specialized networks. We identify multiple
202 capacitors that regulate CGV across multiple environments, one allele behaving as a
203 capacitor and the other as a potentiator. These capacitors are separate from the core network,
204 which is active independently in each of these extreme environments. We identified a single
205 unique network for each environment. These networks maintain tight invariant phenotypes,
206 which are perturbed only in certain allelic combinations, by using either of the following two
207 approaches: maximalist phenotype or minimalist phenotype. These specialized networks
208 probably employ these different capacitors to suppress CGV and as a result these capacitors
209 release CGV, which either have positive effect or negative effect on the phenotype,
210 depending on the kind of network. Our results argue that the regulation of CGV is a
211 fundamental force in evolution of genetic networks underlying all complex traits.

212

213 **RESULTS**

214 **variance QTL mapping identified loci that regulate CGV**

215 QTL mapping allows comparison of effect of two alleles of a locus on a heterogeneous
216 population. In an artificially generated biparental population, like the one used for this study,
217 the majority of the alleles are present at an equal frequency. Therefore, while a difference in
218 the mean of the two populations based on a marker demonstrates the effect of that allele
219 independent of other loci, the difference in variance of the population carrying that allele is a
220 representation of the effect of the allele on phenotypic manifestation of other polymorphisms,
221 or CGV.

222 Using a previously published dataset (Bloom *et al.* 2013) of a recombinant haploid
223 population generated from a biparental cross between a lab strain BY and a vineyard isolate
224 RM11, we carried out linkage mapping to identify genetic loci, which showed an allelic
225 difference on the mean (QTL) and variance (vQTL) of colony size variation. This mapping
226 was done independently in 34 diverse environments, ranging from different carbon sources to
227 oxidative and DNA damaging stresses. QTL were estimated using F-statistic comparisons
228 whereas vQTL were using Brown-Forsythe (BF) test (Figure 1A, see Methods).

229 By comparing the mean and the variance affects of the identified causal variants, we aimed to
230 address the following main questions in this study. Is CGV pervasive in a population such
231 that most loci show a basal difference in variance? Or do specific hubs, *i.e.*, capacitors exist
232 that regulate the release of CGV across traits? And finally, what is the association between
233 phenotypic robustness and CGV; if capacitors do exists, are they present in all environments
234 such that their existence is decoupled from the state of phenotypic robustness of a trait. Or is
235 the role of these capacitors revealed only in certain conditions in which robustness is
236 perturbed?

237 Previous studies have mapped vQTL in segregating populations but their effect sizes were
238 found to be small. Additionally, genetic studies claimed that many loci were capable of
239 regulating phenotypic variance. To estimate the number of loci capable of affecting variance
240 and their effect sizes, the loci were shortlisted separately based on significance and LOD
241 score cutoffs (see Table S1). Based on significance of effects ($P < 0.005$), 44% loci behaved
242 as only QTL and 16% as only vQTL across all environments (Table 1). A large proportion,
243 39%, of markers had a significant effect on both the mean and the variance. This indicated

244 that more than half (55%) of the loci (including only vQTL and QTL+vQTL loci) affected
245 variance. However, as previously observed, the differences in the population mean were more
246 distinguishable than the differences in the population variance. Based on effect sizes (LOD
247 score > 3, permutation $P < 0.01$), 70% of the loci behaved as only QTL, 6% as only vQTL
248 and the remaining (24%) as both (Table 1). Thus while more than 50% of the loci had a
249 significant effect on variance, the effect size was small in most cases. A high overlap was
250 identified between loci regulating the mean and the variance, which indicated that release of
251 CGV was associated with a shift in the population mean. Along with highlighting the ability
252 of commonly studied QTL to regulate population means, this result emphasizes a specific
253 directionality of release of CGV.

254 If presence of capacitors and release of CGV is a common process, uncoupled from inherent
255 robust or buffered state of the population in a given environment, then one would expect a
256 uniform distribution of loci regulating variance across environments. Instead, we observed
257 clustering among these loci regulating phenotypic variance along with mean (QTL+vQTL) in
258 specific environments. A high negative correlation (r^2 = -0.9) was observed between ratios of
259 loci classified as QTL and as QTL+vQTL across environments (Figure 1B). The figure
260 shows that while the majority of environments show exclusive QTL effects, the enrichment
261 of QTL+vQTL loci in certain environments demonstrates that most loci in these
262 environments result in difference in variance along with the mean.

263 **Environment determines the release of CGV**

264 A fundamental question associated with release of variance has been its effect on the average
265 population phenotype, *i.e.*, whether release of variance will generate low or high fitness
266 phenotypes, or both (*i.e.*, CGV will be released equally around the mean). A high number of
267 QTL+vQTL and a few vQTL identified in our analysis established that the release of genetic
268 variance by an allele was exclusively either advantageous or deleterious for the phenotype of
269 the segregants (Figure 2A, see Table S1). While we cannot comment on the effect of colony
270 size on fitness, we identified examples of loci that resulted in either only high or only low
271 mean associated with release of variance indicating that loci were able to exhibit both
272 capabilities (Figure 2A). In very few cases was the release of genetic variance by an allele
273 spread equally around the phenotypic mean. One obvious question, from these observations,
274 is which factors determine the direction of release of variance – does it depend on the locus
275 or is it a property of the environment? Little association was observed between the mean and

276 the variance in different pleiotropic loci across environments. For example, a chrXV
277 (140,012) marker that had a significant effect on the mean and the variance in various
278 environments showed no association between the two (Figure 2B, see Table S2), while the
279 BY allele has a higher mean in $MgCl_2$ and LiCl, and RM allele has a better mean in $MgSO_4$;
280 the RM allele has a higher variance in all these 3 environments suggesting poor correlation
281 (r^2 = 0.3) between the mean and the variance for this particular locus (Figure 2B, see Table
282 S2). However, a strong association between the mean and the variance of loci was observed
283 within each environment (Figure 2B). In environment $MgSO_4$, a strong positive correlation
284 (r^2 = 0.9) was observed such that an allele with a higher mean always had a higher variance
285 independent of the mapped locus. Pair-wise correlation was calculated between the mean and
286 the variance of all significant loci for each environment independently (see Methods). A poor
287 correlation across the environments would have indicated that different loci resulted in
288 random release of variance in both positive and negative directions within an environment.
289 Interestingly, a significant correlation (P < 0.01) was observed in majority of the
290 environments (26/33, Figure 2C). One half of the environments (13/26) showed a strong
291 positive correlation (r^2 > 0.5) indicating that release of variance was, on an average,
292 advantageous for the phenotype. The other half environments showed a negative correlation
293 (r^2 < -0.5), *i.e.*, release of variance resulted in reduced colony size (Figure 2C). This meant
294 that independent of the molecular nature of the regulatory locus or the hidden genetic
295 variation, effect of release of variability on the population mean was strongly dependent on
296 the environment.

297 This prepotency of the environment in determining direction of release of variance raised the
298 possibility of environmental constraint on the nature of released CGV. Do same loci regulate
299 the mean and the variance across different environmental categories (positively versus
300 negatively correlated environments)? If yes, then do these alleles show similar or opposite
301 effects on the regulation of variance between different categories? A comparison of
302 QTL+vQTL effects of a locus across environments was limiting due to higher number of
303 QTL effects than QTL+vQTL or vQTL effects (see Table 1 and Figure 2B). More
304 importantly, alleles of a locus may show similar variance in an environment but behave
305 differently across environments. Trait covariance measures how a population behaves across
306 two environments (Haber and Dworkin 2015). A high covariance means similar phenotype,
307 and similar identity of CGV released in this case, whereas a low covariance indicates
308 differential phenotype of the segregants across the two environments. Therefore, while the

309 two alleles can have similar variance, they may show differential release of CGV, across two
310 environments (Figure 3A). BY allele of the chrXV (160,440) marker showed similar variance
311 in Lactate ($\sigma^2 = 0.88$) and Lactose ($\sigma^2 = 0.69$), much like BY allele of marker chrXIV
312 (681,897) in trehalose ($\sigma^2 = 0.77$) and LiCl ($\sigma^2 = 0.86$). However, the latter allele showed a
313 much lower covariance (*Cov* = 0.13) than the former (*Cov* = 0.84). This indicated that while
314 the RM allele allowed similar phenotypic manifestation of genetic variants in the population
315 in both Lactate and Lactose, BY allele showed differential regulation of these variants across
316 Trehalose and LiCl. Mapping covariance of loci across pairs of environments therefore, will
317 identify loci exhibiting differential CGV across environments, and hence uncover potential
318 capacitors. In addition, covariance mapping will also determine whether allelic bias exists
319 between the ability to release variance, *i.e.*, does one allele always result in release of
320 variance independent of the environment or this ability of an allele is determined by the
321 environment in which the locus is present in. In other words, does an allele behave
322 exclusively as a capacitor or as a potentiator, or can it behave like both depending on the
323 environment? As an alternative to performing a genome-wide covariance analysis, we chose
324 only those loci that had a significant effect (QTL, vQTL or QTL+vQTL) in more than one
325 environment. Forty seven such loci were selected and their covariance was computed across
326 these environmental pairs (see Methods, Table S3). Eighteen loci showed a significant
327 difference in the covariance with 9 being significant across multiple (more than 15)
328 environmental pairs (Figure 3B, Table S3). These 9 loci regulated covariance across multiple
329 environments and therefore were identified as hubs of variance regulation, *i.e.*, capacitors or
330 covariance hubs. The same locus regulated covariance in environments from different
331 categories where the mean and the variance were positively correlated and negatively
332 correlated (Figures 2C, 3C, Table S3). Furthermore, within a locus, while either of the alleles
333 had the ability to result in release of variance across environments, a significant allelic bias in
334 this ability was observed. Among the 5 loci that regulated covariance in more than 15
335 environmental pairs, 4 had a significant allelic bias (Fisher Exact test $P < 0.05$, Figure 3B).
336 This meant that while both the alleles of these loci are capable of functioning as capacitors,
337 one allele tends to contain variance and the other results in its release in majority of the
338 environments. In Figure 3C, Paraquat shows a positive correlation between the mean and the
339 variance whereas Cu shows a positive correlation. However, BY allele of the chrXIII
340 (45,801) marker shows reduced variance in both environments whereas the RM allele shows

341 a higher variance. Hence alleles of a locus tend to behave exclusively either as capacitors or
342 as potentiators, independent of the environment.

343 **CGV as a major contributor to gene-environment interactions, especially antagonistic**
344 **pleiotropy**

345 Environmental influence on the direction of release of variance (a better or a poorer
346 phenotype) and high consistency of the regulation of variance by covariance-regulating
347 pleiotropic hubs have a pronounced ramification on the role of CGV in regulating gene-
348 environment interactions. While CGV explains the high conditional neutrality of genetic
349 effects across environments, we demonstrate that the direction of release of CGV, as
350 determined by the environment, has a strong effect on gene-environment interactions,
351 especially antagonistic pleiotropy. Assume a following scenario where a locus regulates the
352 mean and the variance across two different environments, *i.e.*, there is opposite correlation
353 between the mean and the variance. Since there is a high consistency in regulation of
354 variance, one allele will always result in high variance, while the other allele will always
355 result in lower variance, and hence the high variance allele of this marker will have opposite
356 effects on the mean thus resulting in antagonistic pleiotropy. For example, the locus chrXV
357 (140,012), in Figure 2B, resulted in differential variance and mean in environments $MgCl_2$,
358 LiCl and $MgSO_4$. Because LiCl and $MgSO_4$ have opposite correlations for the mean and the
359 variance, the consistency of RM allele in showing higher variance results in a trade-off in the
360 overall mean effects. This phenomenon is clearly demonstrated by the example in Figure 3C,
361 where BY allele of chrXIII (45,801) marker had better mean in Cu and poorer mean in
362 Paraquat than the RM allele and hence showed antagonistic effects. Therefore, in this
363 example, this antagonism was a result of the consistency of the RM allele in reducing
364 variance and the BY allele in releasing variance. Thus, opposite correlation between the mean
365 and covariance in Paraquat and Cu resulted in antagonistic effect of this locus. Therefore,
366 CGV and its regulation contribute to gene-environment interactions in multiple complex
367 ways.

368 **Two-locus mapping identifies the hidden variants**

369 Identification of loci acting as hubs regulating covariance indicates their ability to function as
370 capacitors. However, to conclusively determine their ability to regulate CGV, identification
371 of the hidden variants is crucial. As previously stated, release of CGV is dependent on gene-
372 gene interactions or epistasis between the stimuli (capacitor or genetic background) and the

373 hidden genetic variants. The conventional use of QTL-QTL mapping is to identify epistatic
374 interactions that contribute to variation in a population. We utilized QTL-QTL mapping to
375 identify the genetic basis and patterns of regulation of released CGV. In addition, we adapted
376 the QTL-QTL mapping technique to perform vQTL-vQTL mapping, which would allow us
377 to identify the loci that show interactive effects of the allelic combinations on the variance of
378 the population instead of the mean. We used QTL-QTL and vQTL-vQTL mapping to
379 understand following points regarding the genetic basis and patterns of regulation of released
380 CGV (see Table S4). Are most genetic interactions environment-specific, such that release of
381 CGV is merely conditionally neutral and environment-specific epistasis? Or do discrete loci
382 exist that behave as capacitors and release CGV across multiple environments? If the
383 covariance hubs, identified previously, indeed function as capacitors, then they should show a
384 high number of genetic interactions across environments. Are there genetic networks that
385 maintain CGV in a population-specific and environment-specific manner? A perturbation of
386 these networks, either due to a rare environment or genetic intermixing, would result in
387 release of CGV. Such a scenario would highlight the role of such network(s) beyond
388 regulation of CGV, in maintaining phenotypic robustness.

389 QTL-QTL and vQTL-vQTL mapping was carried out in an environment dependent manner
390 within all loci that were significant as QTL, vQTL or QTL+vQTL in those environments.
391 Total of 73 significant interactions ($P < 0.1$) were identified of which 18 (24%) were QTL-
392 QTL, 33 (45%) were vQTL-vQTL and 22 (30%) were both QTL-QTL and vQTL-vQTL
393 interactions (Table 2). This substantially high number of vQTL-vQTL interactions, in
394 comparison to proportion of single vQTL (Table 1), indicated their role in contributing to
395 population variation without demonstrating epistasis of the mean effects. Interestingly,
396 amongst the loci showing QTL-QTL interactions, 10% had only QTL effects, whereas 80%
397 were either vQTL or QTL+vQTL (Table 2). In addition, while there was a negative
398 correlation between the total number of QTL in an environment and the number of QTL-QTL
399 (r^2 = -0.46) and vQTL-vQTL (r^2 = -0.37) interactions, a strongly positive correlation was
400 observed between number of QTL+vQTL and number of QTL-QTL (r^2 = 0.64) and vQTL-
401 vQTL (r^2 = 0.65) interactions, indicating that the release of variance was a result of revelation
402 of epistatic effects. In other words, a high number of QTL+vQTL effects is a robust predictor
403 of epistasis in the trait.

404 **Identifying generalist capacitors and specialized genetic networks regulating CGV**

405 By comparing the results of QTL-QTL and vQTL-vQTL interactions across environments,
406 we attempted to distinguish between the three scenarios: environment-specific epistasis,
407 capacitors and networks maintaining robustness. The loci showing interactions, within and
408 across environments, was compiled. A locus that had interactions in two or less than two
409 environments and single interaction per environment was considered as an environment-
410 specific interactor; a locus with interactions in more than two environments was considered a
411 capacitor; and a locus with more than two interactions within an environment was considered
412 a part of a network active within that environment. A total of 73 interactions (QTL-QTL,
413 vQTL-vQTL, QTL-QTL+vQTL-vQTL) were identified, which meant 146 interactors (see
414 Table S5). Amongst these, 29 interactors were involved in environment-specific interactions,
415 55 interactors which attributed to 9 loci showed interactions across diverse environments, *i.e.*,
416 behaved as capacitors. The remaining 62 showed multiple environment-specific interactions.
417 Of the 55 markers that behave as capacitors, 62% marker showed interactions that had a
418 difference in variance (vQTL-vQTL or vQTL-vQTL+QTL-QTL). Interestingly, of the 62
419 markers, which showed environment-specific interaction networks, 85% showed a difference
420 in variance, indicating that these capacitors and networks regulate CGV possibly through
421 higher order interactions. Only 2 loci were involved in multiple interactions across
422 environments (chrVIII and chrXIII). These results showed that environment-specific
423 interactions were rare, and loci which form environment specific networks are different from
424 the ones which behave as capacitors.

425 Epistasis across traits follows a pattern where interaction hubs regulate CGV across multiple
426 environments. Amongst the 9 interaction hubs identified, 7 also behave as covariance hubs
427 indicating that loci that regulate variance across multiple environments do so by interacting
428 with different loci in different environments (Figures 4A, 5A, Table S3). When combined
429 with consistency in direction of covariance, these results demonstrate how CGV is released
430 majorly in the presence of one allele of the capacitor and not the other (Figure 4A). Different
431 genetic variants are released across different environments. The Figure 4A shows that BY
432 allele of the chrXIV (368,185) marker has a higher variance in both 4-HBA and Galactose
433 and the RM allele has a lower variance. In 4-HBA, the chrXIII locus shows a larger effect in
434 the presence of the BY allele than the RM allele (Figure 4B). Similarly, in Galactose, chrXV
435 locus shows a stronger effect on the phenotype in the presence of BY allele than the RM

436 allele (Figure 4C). These results demonstrate how the covariance hubs result in release of
437 differential CGV across different environments.

438 Sixty six markers showed environment-specific networks in 12 environments (see Table S5).
439 Some of these networks were denser than others. These networks showed high
440 interconnectivity and the loci in these networks showed QTL+vQTL effects. Additionally,
441 the interactions showed both QTL-QTL and vQTL-vQTL effects, *e.g.* a network of 4 loci
442 present in Indoleacetic acid (IAA) where all loci show significant interactions with each other
443 (Figure 5B, C). As demonstrated by the dot plots, the allelic combinations differed in mean as
444 well as variance (Figure 5B). As shown above, the differential variance between alleles of a
445 locus is deterministic of allele-specific revelation of effects. This finding (derived from many
446 such examples as one shown in Figure 5, also see Figure 6B) can be extrapolated to state that
447 loci showing vQTL-vQTL interactions harbour higher degree interactions within them, which
448 results in differential variance of the allelic combinations. Indeed, as shown in the Figure 5B,
449 vQTL-vQTL interactions occured when 3 out of 4 biallelic combinations exhibited lower
450 variance, higher mean phenotype, whereas one combination resulted in increase in the
451 variance and reduction in the mean. Similar networks were present in other environments as
452 well, with these environments demonstrating a range of connectivity patterns. While all 4 loci
453 showed similar effects in IAA, networks in other environments have loci that act as hubs
454 showing higher interactions than other members of the network (see Figure 6B). A consistent
455 property of an environment containing such networks is that all loci showing an effect on the
456 phenotype in these environments are a part of this network. They may be present as highly
457 interacting hubs or as interactors of one of these hub loci (see Figure 6B). Additionally, most
458 of the loci of these networks are exclusive to each environment (see Figure 6B). This
459 indicates a revelation of underlying genetic networks in these environments, with rare
460 additive effects of loci. A tempting possibility is that this dense genetic interactome underlies
461 all traits, but is revealed under specific genetic and environmental perturbations.

462

463 **DISCUSSION**

464 The phenomenon of CGV was first proposed over 60 years ago (Waddington 1956). While its
465 existence has been demonstrated in various systems across taxa, the architecture of cryptic
466 genetic variation and its effect on the genetic regulation of complex traits are still unclear
467 (Siegal and Leu 2014). Several questions surround the understanding of regulation of CGV.

468 How is it regulated – by generalist pleiotropic capacitors or by phenotype-specific networks?
469 What effect does it has on the phenotype, in other words, what is the distribution of
470 deleterious versus advantageous CGV? Can the effect of release of CGV on the phenotype be
471 predicted? Is the architecture of the hidden genetic variants different from that of the additive
472 loci (Gibson and Reed 2008)? Studies have demonstrated both detrimental and beneficial
473 effects of CGV on various phenotypes (Jarosz *et al.* 2010). In addition, contribution of
474 various phenomena *viz.* robustness, canalization, buffering and capacitance to CGV, further
475 complicates its mechanistic understanding (Gibson and Dworkin 2004). We used an
476 integrative approach of mapping loci regulating mean, variance and covariance and their
477 epistatic regulation to understand the underlying genetic framework of CGV. Comparing
478 genetic regulation of multiple environments allowed us to comprehensively study the
479 regulation of CGV and the integrated co-existence of conventionally identified loci and CGV.
480 We propose the following model based on our results (Figure 6A).

481 A population can exist in different states based on its genetic diversity and the surrounding
482 environment. While we do not understand the underlying mechanistic differences between
483 these states, they appear to be a gradient of the internal buffering states or phenotypic
484 robustness (of heritable variation) of the population. Towards the left end (gray) are states
485 where genetic variation is majorly additive in nature, with a few epistatic interactions such
486 that most loci have independent effects with difference in the population mean. Some loci do
487 show pleiotropic effects in these states but only affect the mean in different environments.
488 For the biparental population used in this study, majority (more than half) of the
489 environments are confined to this side of the spectrum. This result is substantiated by
490 mapping of loci with predominantly additive effects and limited identification of epistasis in
491 different studies (Carlborg and Haley 2004; Bloom *et al.* 2015). Additionally, this explains
492 the low effect of variance QTL identified in multiple studies. Moving towards the right side
493 (blue)along this gradient, the population state gets altered such a way that the underlying
494 genetic interconnectivity, and hence CGV, gets revealed (Carlborg and Haley 2004; Mackay
495 2014; Siegal and Leu 2014; Fares 2015). This perturbation appears to happen in rare
496 environments (Kitano 2004) and uncovers both kinds of genetic buffering mechanisms –
497 capacitors, which regulate CGV across multiple environments and environment-specific
498 genetic networks. Results from a recent study, which has identified capacitors and
499 potentiators of bristle numbers in *D. melanogaster*, support our model, thus suggesting its
500 universality (Takahashi 2015). Not just regulation of CGV, but these two mechanisms

501 together form the core genetic regulation in these environments (Félix and Barkoulas 2015).
502 The unusualness of these environments provides support for the Highly Optimized Tolerance
503 (HOT) hypothesis, which states that systems evolved to be robust against most common
504 perturbations, show high vulnerability to rare conditions (Carlson and Doyle 2002).

505 The capacitors identified by our study regulate the release of CGV across multiple
506 environments, as demonstrated both by their ability to regulate covariance as well as by
507 multiple epistatic interactions exhibited by them across environments. A few molecular
508 examples of such capacitors have been identified including *HSP90* (Rutherford and Lindquist
509 1998), *HZT1* (Richardson *et al.* 2013) and *IRA2* (Taylor and Ehrenreich 2015). These genes
510 have been shown to result in revelation of the hidden variants upon genetic or environmental
511 perturbations. However, a discrepancy exists between the theoretical understanding of
512 evolutionary capacitors and functions of these genes, *HSP90* and *HZT1*, since CGV is
513 released both in their presence and absence. While the released CGV depends on their
514 functional state, thus demonstrating their ability to show high epistasis, this argues against
515 their role in evolution towards maintenance of phenotypic robustness. Such disparity in their
516 roles questions the overlap between robustness and CGV regulation. While the capacitors
517 identified in our study show a similar behavior, *i.e.*, both alleles have the capability to result
518 in release of variance; they show a strong allelic bias. In majority of the cases one allele
519 allowed the interacting locus to manifest phenotypically whereas the other allele suppressed
520 its effect such that one allele behaved as a potentiator whereas the allele behaved as a
521 capacitor. This along with the enrichment of such capacitors in only certain environments
522 indicates a link between release of CGV and robustness of the phenotype.

523 A classic argument against an association of such loci, which regulate epistasis in standing
524 genetic variation, with phenotypic robustness and buffering has been a lack of demonstration
525 of their ability to accumulate new mutations. These arguments propound that CGV is
526 independent of the state of phenotypic robustness (Paaby and Rockman 2014). While we
527 have focused only on standing genetic variation in this study and hence cannot comment on
528 mutational robustness, we observed a strong bias in the ability of a few environments to
529 activate these capacitors. These capacitors have pleiotropic effects across all the
530 environments (Cassidy *et al.* 2016) but they regulate variance only in the few environments
531 found on the right side of the gradient and act as conventional QTL for the environments
532 towards the left (Figure 6A). While the effect of these capacitors on novel mutations, and
533 hence mutational robustness, cannot be demonstrated, our results confidently show that the

534 ability of these loci to behave as capacitors is dependent on the environment and hence may
535 be a representation of the buffered state of the environment (Fares 2015). Our results,
536 therefore, argue in favor of evolution of these capacitors to maintain CGV (Elena and Lenski
537 2001).

538 In these specific environments, along with increased prevalence of capacitors, we identified
539 highly interconnected networks (Figure 6B). These genetic networks were specific to each
540 environment. The pleiotropic capacitors showed an effect in these environments but they
541 were just one the interactors – either a part of the network or an interactor of the hub gene in
542 the network, but never forming the core themselves (Figure 6A,B). An interesting property in
543 these environments was the extensive cross talk that resulted in a single specific network in
544 each environment, *i.e.*, almost all loci active in a particular environment form a part of this
545 single network. The high number of epistatic interactions in this extreme right of the
546 spectrum, and increased differential regulation of population variance at both single locus and
547 two-locus levels indicates prevalence of higher order epistasis (Figure 6A,B). While we do
548 not have the power to confidently demonstrate such high order interactions, multiple epistasic
549 interactions between a few loci and their vQTL-vQTL mapping plots suggest their presence
550 (see Figure 5B,C). These results support the predictions of high prevalence of such higher
551 order interactions (Carlborg *et al.* 2006; Taylor and Ehrenreich 2014). This high
552 interconnectivity is the reason why we observe so few additive QTL in the left side of the
553 gradient with the majority of loci behaving as QTL+vQTL.

554 As described above, while the pleiotropic capacitors are also a part of these interactions, they
555 do not form the core network. A reason for this exclusion can be that these networks could
556 have evolved in response to specific selection pressures to reach phenotypic optimum for a
557 particular phenotype, and the pleiotropic nature of the capacitors would implement a
558 constraint on their ability to evolve or mold to a specific environment. These results indicate
559 that a possible reason for the evolution of pleiotropic genes could have been the regulation of
560 this CGV across environments. Furthermore, modularity of the interactome identified
561 recently in disease networks (Vidal *et al.* 2011; Menche *et al.* 2015) to regulate specific
562 diseases could be a result of evolution of these environment or phenotype specific hubs,
563 which maintain robustness for specific phenotypes by genetic redundancy (Kafri *et al.* 2009).
564 At a molecular level this indicates that these specific networks, which maintain robustness
565 and contain CGV form the core regulation in each environment. A perturbation in them
566 results in release of CGV, probably by impinging on these capacitors. Our results argue that

567 such an interconnected system probably underlies the regulation of all complex traits but
568 unveils itself only upon certain environmental and genetic perturbation. Different
569 environments or phenotypes have different core networks (Chari and Dworkin 2013), which
570 impinge on these capacitors and regulate phenotypic variation and robustness (de Visser *et al.*
571 2003; Chevin *et al.* 2010).

572 Along with high epistasis or gene-gene interactions (Mackay 2014), a major consequence of
573 CGV is high gene-environment interactions, since these hidden variants show their effects in
574 certain environments and not in others. While this would explain the high conditional
575 neutrality in genetic effects across environments identified in various gene-environment
576 interaction studies, it does not justify the observed environmental constraint on the direction
577 of release of variance (see Figure 2B). Our results show that CGV is released in only one
578 direction in each environment and QTL-QTL and vQTL-vQTL mapping has shown that one
579 single network is active in each environment. Therefore, one can surmise that the hidden
580 variation is released in only one direction by a particular network. Based on our results, we
581 propose that a particular network, evolved to maintain optimum phenotype, can curb
582 expression of phenotypic variation in two ways – the maximalistic approach or the
583 minimalistic approach. The maximalistic approach would be evolution of networks that
584 maintain high flux through the pathway(s) contributing to the phenotype such that the default
585 state is always on. In this scenario, any genetic variant that results in a reduced flux, and
586 hence lower phenotype, will not have much effect since there will be an excess of
587 transcriptional or biochemical signal. As a result, such variants will get accumulated due to
588 lack of purifying selection on them, whereas a variant that increases the flux will get fixed
589 and become a part of the network. Perturbation of this network will, therefore, only release
590 variants with a poorer phenotype. The alternate minimalistic approach would describe a
591 default state with the minimum possible flux, such that the network maintains the pathway(s)
592 in a practically shutdown state. Since the upstream signal is off or minimal, the downstream
593 genetic variants that increase the flux will not be able to show their effects and hence get
594 fixed. Consequently, a breakdown of this network will reveal genetic variants that increase
595 the phenotype. Our conjecture is that modules or specialized network may have evolved this
596 way to contain CGV in the most optimal manner. While this regulation may happen at
597 various levels (transcription, translation or for metabolites) and differ for different
598 phenotypes and environments, it would follow either of the two above approaches.

599 Along with providing molecular insights into genetic regulation of various traits, this
600 directionality of the networks has strong implications on gene-environment interactions,
601 especially antagonistic pleiotropy. While the core network loci are environment-specific, the
602 generalist capacitors are common across different environments. Our results show that the
603 abundant antagonistic pleiotropy of the mean effects of these loci is not due to opposing
604 molecular effect of the locus on the two environments but majorly due to the opposing
605 constraints of the environment on direction of released CGV (see Figure 3). Depending on
606 the kind of network that impinges on the capacitor, the released CGV will either increase or
607 decrease the phenotype. Therefore, along with regulating condition specificity of the effects
608 of loci, regulation of CGV can result in trade-offs in the effect of loci across different
609 environments. In our previous paper, we reported high antagonistic pleiotropy of a locus on
610 chrXV containing the gene, *IRA2* (Yadav *et al.* 2015). *IRA2* is an inhibitor of the highly
611 conserved Ras/PKA pathway, which we demonstrated to show high diversity in natural
612 populations of *S. cerevisiae* adapted to diverse ecological and geographical niches.
613 Investigation of QTL and vQTL revealed that the loci containing *IRA2* is a covariance hub
614 and hence a capacitor (see Table S3). This is supported by a recent study, which
615 demonstrated role of *IRA2* in regulating cryptic variation in colony morphology trait by
616 disruption of transcriptional silencing of one or more genes that affect the trait (Taylor and
617 Ehrenreich 2015). Comparing the mean and the variance revealed that the antagonistic effect
618 in different environments is a consequence of differential robustness of the two alleles of
619 *IRA2*, which may be the primary reason underlying their role in adaptation. This has profound
620 significance on the increasing identification of antagonistic effects in various diseases
621 causing alleles (Carter and Nguyen 2011). Our study indicates that their opposing effects are
622 potentially a result of their differential robustness in different populations and lifestyles. This
623 inference will be crucial in understanding their pleiotropic effects as well as their molecular
624 mechanisms.

625 Our study provides a detailed understanding of the architecture of CGV affecting yeast
626 growth across multiple environments. In doing so, we have uncovered patterns and governing
627 principles which can be used to predict existence and behavior of CGV. This underlying
628 architecture has the potential to elucidate certain fundamental, hitherto unclear, phenomena in
629 quantitative biology – varying contribution of epistasis in different traits, pleiotropy, network
630 modularity, environment and genetic background dependence of causal loci. Revisiting the
631 already existence datasets of breeding traits and human diseases in the light of conclusions

632 from this study can provide better insights into their genetic regulation and has the potential
633 to solve the mystery of missing heritability (Eichler *et al.* 2010; Nelson *et al.* 2013).
634 Identification of regulation of CGV can provide better comprehension of the molecular
635 functioning and evolution of the modules regulating various traits and disease.

636

637 **METHODS**

638 **Dataset**

639 The raw growth data analysed in this study was derived from a study by Bloom *et al*. (2013),
640 in which the experimental procedures are described in detail. The data we used was generated
641 for 1,008 segregants derived from a cross between *S. cerevisiae* strains BY (a laboratory
642 strain) and RM11-1a (a wine isolate, indicated as RM). These segregants were grown in 46
643 different conditions and phenotyped for colony size of which 34 conditions were considered
644 in this study (see Table S1, see Files S1 and S2 for more information). A total of 11,623
645 markers were considered.

646 **QTL and vQTL mapping**

647 The single environment QTL mapping was carried out as described previously (Bhatia *et al.*
648 2014). In brief, the R/qtl package (Broman *et al.* 2003; Broman and Sen 2009) was used to
649 identify QTL separately for colony size in each environment. QTL were identified using the
650 LOD score, which is the $\log_{10}$ of the ratio of the likelihood of the experimental hypothesis to
651 the likelihood of the null hypothesis (Broman and Sen 2009). An interval mapping method
652 ("scanone" function in R/qtl) was used to compute this LOD score using the Haley-Knott
653 regression algorithm (Broman *et al.* 2003).

654 The following formula was used to calculate the F-score, which was further used to derive the
655 LOD score. At a particular marker, let segregant *i*'s phenotypic value be y_{ij} where j can
656 take two values ($j=1$: BY allele and $j=2$: RM allele).

657
$$F=\frac{\sum_{j=1}^{k} n_j\left(\bar{y}_j-\bar{y}\right)^2 \Big/ (k-1)}{\sum_{j=1}^{k}\sum_{i=1}^{n_j}\left(y_{ij}-\bar{y}_j\right)^2 \Big/ (n-k)}$$

658 Here, N is the total number of segregants, n_1 and n_2 are the number of segregants having
659 the BY and RM allele respectively ($k = 2$) and y_j is the genotypic mean of allele *j*.

660 Let *df* denote the degrees of freedom (*df* = 1 for a backcross and *df* = 2 for an intercross). The
661 LOD score is accordingly derived as follows:

662 $$LOD = \frac{n}{2}\log_{10}\left[F\left(\frac{df}{n-df-1}\right)+1\right]$$

663 Under the null hypothesis, there is no significant difference in the means at the marker under
664 consideration while under the alternative hypothesis, there is a presence of a QTL.

665 To estimate the difference in phenotypic variance between the two genotypic groups, *i.e.*, to
666 identify vQTL in each environment, the standard Brown-Forsythe (BF) statistic (Rönnegård
667 and Valdar 2011; Lee *et al.* 2014) and the corresponding LOD score were calculated for each
668 genetic marker in each environment (see Files S1 and S2). The BF test is equivalent to an F-
669 test performed on the deviations of the phenotypic values from their respective genotypic
670 medians (or the means). Hence, under the alternative hypothesis, the phenotypes of the two
671 alleles reveal a difference in the variance.

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

675 $$z_{ij} = \left|y_{ij} - \tilde{y}_j\right|$$

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

677 $$F = \frac{(N-p)}{(p-1)}\frac{\sum_{j=1}^{p} n_j(\tilde{z}_{.j} - \tilde{z}_{..})^2}{\sum_{j=1}^{p}\sum_{i=1}^{n_j}(z_{ij} - \tilde{z}_{.j})^2}$$

678 Here, N is the total number of segregants, n_1 and n_2 are the number of segregants having
679 the BY and RM allele respectively ($p = 2$). In order to estimate the effects of vQTL in the
680 same order as in QTL, LOD scores were computed as described previously (Broman and Sen
681 2009).

682 To establish the statistical significance of the putative QTL and vQTL, *P* values were
683 computed using a genome-wide permutation test of 1,000 permutations, where the null
684 distribution consisted of the highest genome-wide LOD score obtained from each
685 permutation (Broman *et al.* 2003). A LOD score cut off of greater than 3.0 and a *P* value cut
686 off of less than 0.01 was considered.

687 **Comparing QTL and vQTL**

688 To estimate pleiotropy, we divided the genome into 20kb non-overlapping bins (Table S3).
689 The bins containing two or more QTL or vQTL significant ($P < 0.01$) in different
690 environments were considered as pleiotropic bins. The first markers of each of these
691 pleiotropic bins, used as representative of the bins, were collated to represent the set of
692 pleiotropic markers (Table S3).

693 **Calculating correlation between mean and variance**

694 To calculate environment-specific correlation (for Figure 2C), difference in the mean (mean
695 BY – mean RM) and difference in the variance (var BY – var RM) was calculated for all
696 significant loci within each environment. Pearson's correlation between these two parameters
697 was calculated for each environment.

698 **Covariance across environmental pairs**

699 To assess the differential covariance of a locus across multiple pairs of environments, we
700 considered the collated set of pleiotropic markers for our study (Table S3). To quantify the
701 differential covariance across a pair of environments, a Deming regression was calculated
702 between the phenotype values of the chosen pair of environments for each allele, using R
703 package 'mcr'. Deming regression, which minimizes errors in multiple dimensions
704 simultaneously, served as a suitable measurement error model for assessing buffering across
705 two or more environments. Unlike simple least squares regression, Deming regression
706 accounts for deviations in observations on both the *x*- and the *y*- axis. It seeks to find the line
707 of best fit

708 $$y^* = \beta_0 + \beta_1 x^*$$

709 such that the below weighted sum of squared residuals is minimized,

710 $$\frac{1}{\sigma_\varepsilon^2}\sum_{i=1}^{n}\left(\left(y_i-\beta_0-\beta_1 x_i^*\right)^2+\delta\left(x_i-x_i^*\right)^2\right)$$

711 where, the estimate of δ is the ratio of the two variances.

712 Since both the phenotypic values (*x* and *y*) are normally distributed across their respective
713 axes, Deming regression, serves as a suitable measurement-error model for assessing
714 buffering across two environments. Two Deming regression models were fitted for each
715 environment pair corresponding to the two alleles.

716 A Student's t-test was performed between the deviations of the phenotypic values from the
717 Deming fit of the BY and RM alleles ($P < 0.05$). This sign of the t-test value represented the
718 allele that had higher mean of deviations, *i.e.*, lower covariance. If t-test value was positive,
719 then the mean deviation of BY allele was greater than that of the RM allele and vice versa.
720 All the significant markers and their corresponding environmental pairs along with mean
721 deviations of the alleles and independent mean and variance of both the alleles and both the
722 environments are listed in Table S3. A locus with significantly different allelic covariance
723 across more than 15 environmental pairs was considered pleiotropic. For each of these loci,
724 total number of positive and negative t-test statistic were compiled. A Fisher's Exact test of
725 random distribution of positive and negative t-test statistic was done each locus, normalized
726 for the number of environmental pairs. A *P* value cut-off less than 0.05 was considered
727 significant.

728 **QTL-QTL and vQTL-vQTL mapping**

729 A QTL-QTL interaction occurs when an effect of a QTL at a single locus depends on another
730 locus. We used a QTL-QTL mapping technique described previously (Bhatia *et al.* 2014). In
731 brief, we used a custom Python script, from Bhatia *et al*. (2014) to compute LOD scores for
732 pairwise comparisons among a set of selected markers. These markers were selected using a
733 QTL *P* value cut-off of 0.01 in each environment. For the QTL-QTL mapping, *P* values were
734 computed using a permutation test (10,000 permutations), where the null distribution
735 consisted of the highest LOD score obtained among all pairwise comparison for each
736 permutation of the phenotype. The following hypotheses were compared:

737 $$H_I : y_i = \mu + \beta_1 g_{1i} + \beta_2 g_{2i} + \gamma g_{1i} g_{2i} + \epsilon_i$$

738 $$H_A : y_i = \mu + \beta_1 g_{1i} + \beta_2 g_{2i} + \epsilon_i$$

739 Here, g_{1i} and g_{2i} are the binary variables that specify the genotype at the two loci, μ, β_1,
740 β_2 and γ are inferred from the data using maximum likelihood. The parameters β_1 and β_2
741 quantify the individual effect of each QTL, and γ quantifies the effect of the QTL-QTL
742 interaction. See Files S1 and S2 for details of calculation of the interaction LOD (LOD_i)
743 score.

744 Apart from the QTL-QTL mapping described previously (Bhatia *et al.* 2014), we mapped
745 variance-controlling interactions by mapping vQTL-vQTL interactions, which occurs when
746 the phenotypic variance at one locus depends on the genotype at another locus. The
747 hypothesis testing for vQTL-vQTL interactions was done in the same way as QTL-QTL
748 interactions except that instead of scaled values of colony size as the phenotypic values,
749 deviations from the mean were used as the phenotype for each allelic combination (see
750 method for vQTL mapping).

751 To increase power to identify QTL-QTL and vQTL-vQTL interactions, for each
752 environment, genetic loci significant in either QTL or vQTL or QTL+vQTL mappings were
753 collated for each environment (Table S1). To compile QTL-QTL and vQTL-vQTL
754 interaction results, loci within a 50kb linkage interval were considered the same. The *P*
755 values were computed using a permutation test of 10,000 permutations with the phenotype
756 data shuffled relative to the genotype data.

757

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762

763 **AUTHOR CONTRIBUTIONS**

764 Conceived and designed analysis: AY HS; Analysed data: KD AY HS; Wrote the paper: AY
765 HS

766

767

768 **TABLES**

769 **Table 1 – QTL, vQTL and QTL+vQTL**

Category	No. of QTL	No. of vQTL	No. of QTL+vQTL
Based on $P < 0.005$	39 (16.6%)	104 (44.3%)	92 (31.1%)
Based on LOD > 3.0	19 (6.4%)	212 (71.6%)	65 (22.0%)

770

771

772 **Table 2 – Two-locus interaction mapping**

Interactions ($P < 0.1$)	QTL-QTL	vQTL-vQTL	QTL-QTL+vQTL-vQTL
	18 (24.7%)	33 (45.2%)	22 (30.1%)

773

774

775 **FIGURE LEGENDS**

776 **Figure 1: QTL and vQTL mapping**

777 (A) Schematic showing three categories of QTL mapped. QTL has a significantly different
778 allelic mean but a non-significant difference in allelic variance; vQTL has a non-significant
779 mean difference but significantly different variance; QTL+vQTL has both significantly
780 different allelic mean and variance.

781 (B) Distribution of loci mapped in each environment as QTL (green) and QTL+vQTL (dark
782 pink) in the segregating population. The y-axis is proportion of QTL or vQTL in each
783 category. The x-axis is different environments arranged by decreasing proportion of QTL.
784 Permutation value cut off < 0.01. See Table S1 for details.

785

786 **Figure 2: Environment and locus dependence of variance**

787 (A) Representative frequency distributions of three loci showing directionality of release of
788 variance. Blue distribution is of segregants with BY allele and red is for segregants with RM
789 allele. 4-NQO [chrXII (207,340)] marker shows release of variance of the BY allele in the
790 negative direction (mean RM > mean BY); Lactose [chrXIV (376,315)] marker shows equal
791 variance of the two alleles; $MgSO_4$ [chrVII (187,538)] marker shows release of variance of
792 BY allele in the positive direction (mean RM < mean BY). The QTL are indicated as
793 chromosome number followed by marker position in bp within brackets.

794 (B) Environment and locus dependence of the mean and the variance of loci. The horizontal
795 arm of the L-shaped grid represents multiple loci L1-Ln significant in environment E1. The
796 corresponding graph shows the mean and the variance of BY and RM alleles of all markers
797 (x-axis) in $MgSO_4$. The vertical grid represents pleiotropic effect of the locus L1 in multiple
798 environments E1-En and corresponding graph shows mean and variance of the marker chrXV
799 (140,012) in various environments (x-axis).

800 (C) Correlation between the mean and the variance of all QTL in each environment.
801 Correlations less than -0.5 (red, $P < 0.05$) represent environments that have a negative
802 association between the mean and the variance. Correlations more than 0.5 (yellow, $P < 0.05$)
803 represent environments that have a positive association between the mean and the variance.
804 Environments with no directional release of variance (correlations between ±0.5) are

805 represented as blue. The y-axis is correlation between mean and variance; dashed lines mark
806 ±0.5 correlation value.

807

808 **Figure 3: Allelic bias in across environment covariance**

809 (A) Covariance of normalized growth phenotype of the BY (blue) allele of chrXV (160,440)
810 marker in Lactose and Lactate. Covariance of normalized growth phenotype of the BY (blue)
811 allele of chrXIV (481,897) marker in Trehalose and Lithium chloride (LiCl). The mean and
812 the variance of each allele in each environment are indicated in the box. The QTL is indicated
813 as a chromosome number followed by the marker position in bp within brackets.

814 (B) Frequency distribution of number of positive and negative values of Deming regression t-
815 test (see Methods) across pleiotropic covariance loci (x-axis). The t-test value is positive
816 where Cov(BY) > Cov(RM) and negative for Cov(BY) < Cov(RM). The y-axis indicates
817 number of environmental pairs across which the locus showed a significant difference in
818 covariance. A significant Fisher Exact test ($P < 0.05$) is indicated by a star. The QTL is
819 indicated as a chromosome number followed by the marker position in bp within brackets.

820 (C) Covariance of normalized growth phenotype of the BY (blue) and the RM (red) allele of
821 chrXIII (45,801) marker in Paraquat and Cu (Copper). The mean and the variance of each
822 allele in each environment are indicated in the box. Paraquat shows a positive correlation
823 between the mean and the variance whereas Cu shows a negative correlation (Figure 2C).
824 The QTL is indicated as a chromosome number followed by the marker position in bp within
825 brackets.

826

827 **Figure 4: Release of CGV through QTL-QTL interactions**

828 (A) Covariance of normalized growth phenotype of the BY (blue) and the RM (red)
829 segregants for chrXIV (368,185) marker in 4-HBA and Galactose. The mean and the variance
830 of each allele in each environment is indicated in the box. The axes are normalized growth of
831 segregants in the two environments indicated.

832 (B) QTL-QTL interaction between chrXIV (368,185) - chrXIII (46,758) in 4-HBA.

833 (C) QTL-QTL interaction between chrXIV (368,165) - chrXV (555,452) in Galactose.

834 The QTL is indicated as a chromosome number followed by the marker position in bp within
835 brackets. For (B, C), the x-axis is normalized growth of segregants in the environment and
836 the y-axis of number of segregants. Dash lines in segregant distributions (B, C) indicate the
837 means of the distributions. The biallelic marker segregant distributions (in the QTL marker
838 order written above the plots) are indicated as BB (light blue), BR (light brown), RB (dark
839 green) and RR (purple). Inset plots show the average distributions of the first marker (BY
840 (blue) and RM (red) alleles). See Table S4 for details.

841

842 **Figure 5: QTL-QTL interaction hubs and networks**

843 (A) QTL-QTL interactions between various markers shown as connected links. The chrII
844 (245,879) marker (red) has multiple QTL-QTL interactions (deep red) for growth in same
845 environment (Congo red). The chrXIV (466,590) marker (green) has 6 environment-specific
846 QTL-QTL interactions (4-NQO = dark green, Formamide = deep purple, Indoleacetic acid
847 (IAA) = light blue, Lithium chloride (LiCl) = deep orange, Trehalose = deep yellow, Xylose
848 = orange). Other QTL-QTL interactions are indicated as light grey links. Genomic interaction
849 maps made using Circos (Krzywinski *et al.* 2009). See Table S4 for data.

850 (B) Scatter plots showing examples of QTL-QTL interactions of four markers [chrIII
851 (191,928), chrIV (997,621), chrVIII (101,016), chrXIV (466,105)] in Indoleacetic acid
852 (IAA). The biallelic marker segregant distributions (in the QTL marker order written above
853 the plots) are indicated as BB (red), BR (yellow), RB (green) and RR (blue) on x-axis. The
854 mean and the variance of each allelic pair is indicated in the box with allelic pair with most
855 variance indicated in red. The y-axis is normalized growth phenotype.

856 (C) Schematic representation of genetic network of four loci, indicated in (B) above, showing
857 QTL-QTL interactions in Indoleacetic acid.

858

859 **Figure 6: Genetic architecture of CGV**

860 (A) A model showing gradient of phenotypic buffering from gray (left side) to blue (right
861 side). Different environments fall in different positions along this gradient. The left side
862 indicates robust buffering in the population such that most loci have additive independent
863 effects with a few epistatic interactions. On the right side, are populations with revealed CGV

that exposes the underlying genetic networks present in different environments. Different environments are represented by different colors. The white nodes refer to independent additive loci, gray nodes refer to loci which are a part of different genetic networks. The gray nodes with thick borders denote capacitors. Most of the environments fall on the left side of the gradient such that the majority of the QTL identified in different studies are additive with a few epistatic interactions. As the underlying buffering state of the population gets perturbed, due to either genetic or environmental perturbations, it moves towards the right side of the gradient. This perturbation reveals the underlying genetic network, which regulates phenotypic variation such that all loci are part of this highly interconnected network. These networks are environment-specific interspersed with generalist capacitors which regulate buffering and epistasis across different environments.

(B) Examples of specific genetic networks identified in different environments using QTL-QTL and vQTL-vQTL interaction mapping. The potential capacitors identified through covariance mapping (see Figure 3B) are highlighted as nodes with black outlines. See Tables S4, S5 for details.

880 **SUPPORTING INFORMATION LEGEND**

881 **File S1**: Scripts and datasets for QTL, vQTL, Deming regression and QTL-QTL, vQTL-
882 vQTL interaction mapping.

883 **File S2**: Supplementary methods.

884 **Table S1**: QTL and vQTL mapping

885 QTL test and vQTL test refers to the F-test and BF-test statistics respectively, and QTL *P*
886 value and vQTL *P* value refers to the *P* values of the corresponding tests. Test cutoff of >3
887 and *P* value cutoff of <0.005 was used to identify significant markers. 4-HBA refers to 4-
888 Hydroxybenzaldehyde; 4-NQO is 4-Nitroquinoline; 5-FC is 5-Fluorocytosine; 5-FU is 5
889 Fluorouracil; 6-AU is 6-Azauracil.

890 **Table S2**: Mean and variance of significant QTL and QTL+vQTL

891 **Table S3**: t-test of Deming regression to compare trait covariance

892 t-test refers to t-test statistic of Student's t-test performed on Deming regression values of the
893 two alleles of a maker across environment 1 and 2. meanRM and meanBY refers to mean of
894 the regression values of RM and BY alleles, respectively, across environment 1 and 2.
895 meanRM_env1 and varRM_env1refers to mean and variance of the RM allele in environment
896 1. Same nomenclature applies to other alleles and environments.

897 **Table S4**: QTL-QTL and vQTL-vQTL mapping

898 Permutation *P* value cutoff of < 0.1 was considered. Sheet 1 contains results of QTL-QTL
899 mapping and Sheet 2 contains results of vQTL-vQTL mapping. Sheet 3 shows the overlap of
900 the Sheet 1 and Sheet 2. In Sheet 3, markers with a 50kb interval were considered the same to
901 estimate the overlap between the two kinds of mapping. A significant QTL-QTL or a vQTL-
902 vQTL interaction is indicated by 1.

903 **Table S5**: Classifying interacting loci in specific gene-gene interactions, capacitors and
904 environment-specific genetic networks.

905

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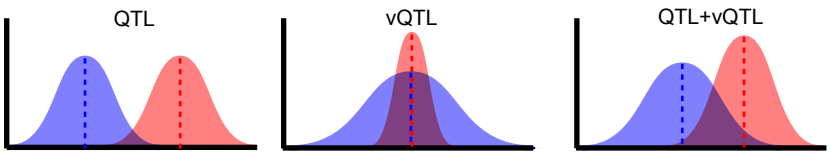
Figure 1

A


QTL
vQTL
QTL+vQTL

B

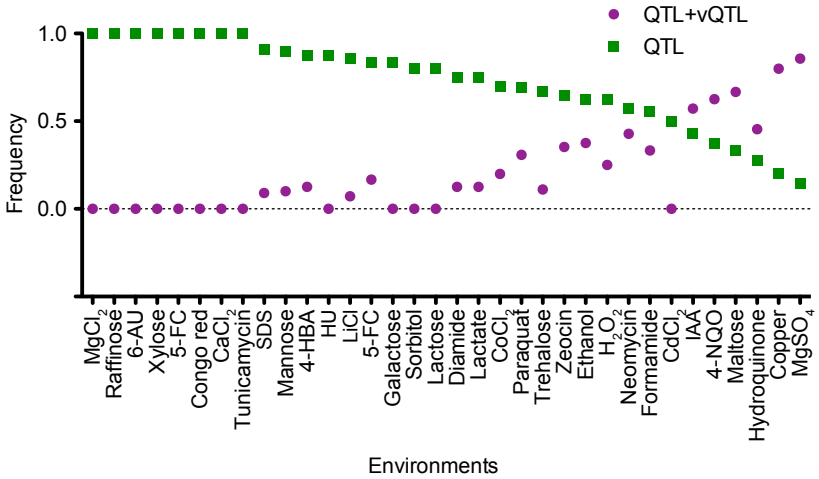
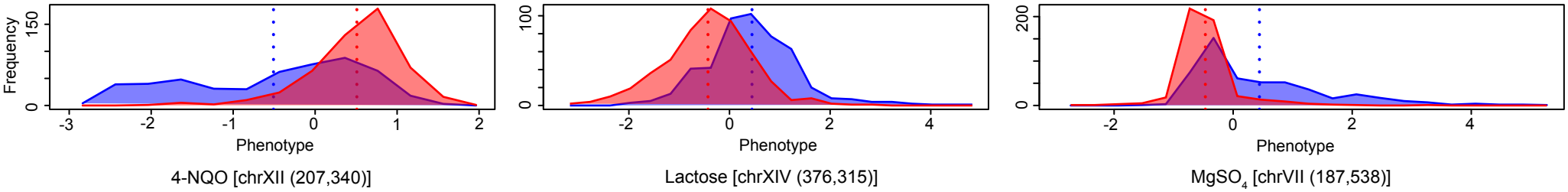
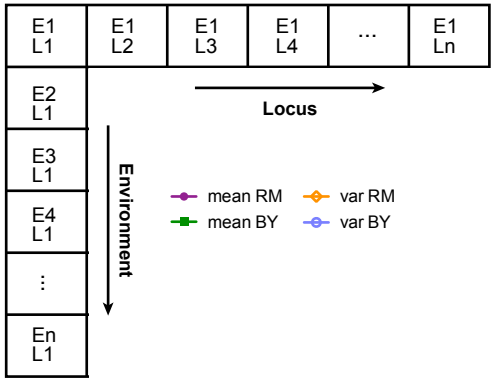

QTL+vQTL
QTL
1.0
0.5
0.0
Frequency
MgCl2
Raffinose
6-AU
Xylose
5-FC
Congo red
CaCl2
Tunicamycin
SDS
Mannose
4-HBA
HU
LiCl
5-FC
Galactose
Sorbitol
Lactose
Diamide
Lactate
CoCl2
Paraquat
Trehalose
Zeocin
Ethanol
H2O2
Neomycin
Formamide
CdCl2
IAA
4-NQO
Maltose
Hydroquinone
Copper
MgSO4
Environments

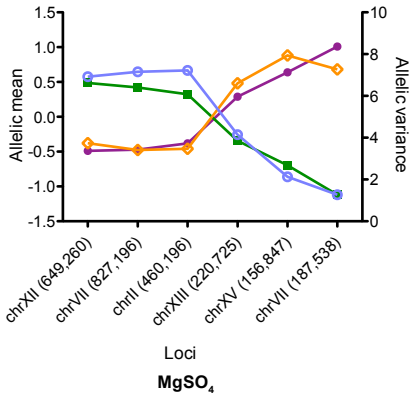
Figure 2

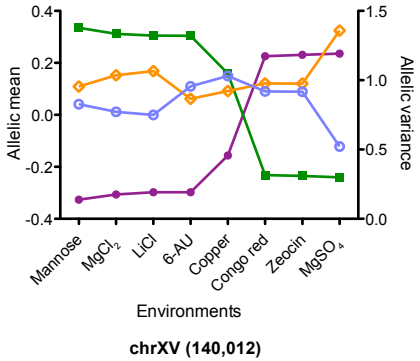
A


Frequency
Phenotype
4-NQO [chrXII (207,340)]
Lactose [chrXIV (376,315)]
MgSO4 [chrVII (187,538)]

B


E1 L1
E1 L2
E1 L3
E1 L4
...
E1 Ln
E2 L1
E3 L1
E4 L1
...
En L1
Locus
Environment
mean RM
mean BY
var RM
var BY


Allelic mean
Allelic variance
chrXII (649,260)
chrVII (827,196)
chrII (460,196)
chrXIII (220,725)
chrXV (156,847)
chrVII (187,538)
Loci
MgSO4


Allelic mean
Allelic variance
Mannose
MgCl2
LiCl
6-AU
Copper
Congo red
Zeocin
MgSO4
Environments
chrXV (140,012)

C

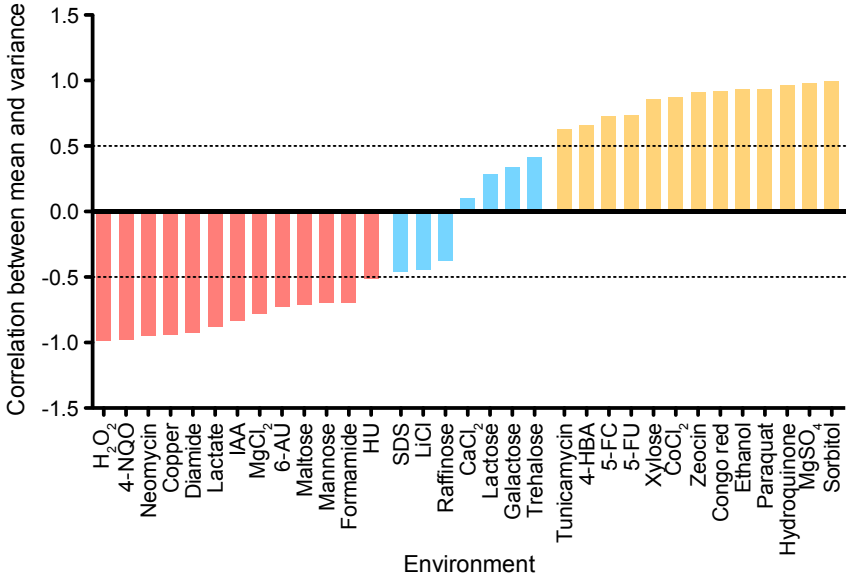

Correlation between mean and variance
H2O2
4-NQO
Neomycin
Copper
Diamide
Lactate
IAA
MgCl2
6-AU
Maltose
Mannose
Formamide
HU
SDS
LiCl
Raffinose
CaCl2
Lactose
Galactose
Trehalose
Tunicamycin
4-HBA
5-FC
5-FU
Xylose
CoCl2
Zeocin
Congo red
Ethanol
Paraquat
Hydroquinone
MgSO4
Sorbitol
Environment

Figure 3

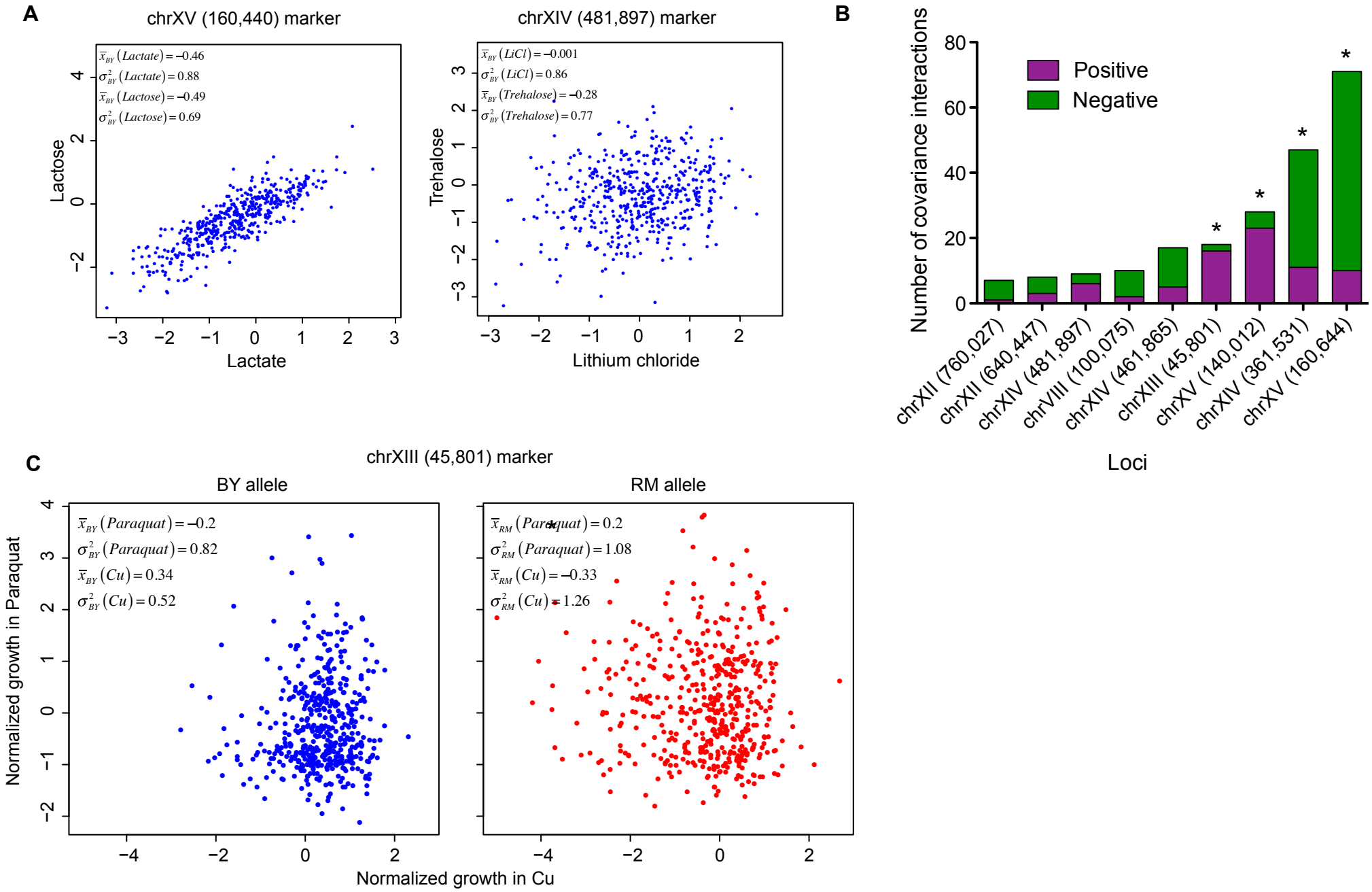

A
chrXV (160,440) marker
$\bar{x}_{BY}(Lactate) = -0.46$
$\sigma^2_{BY}(Lactate) = 0.88$
$\bar{x}_{BY}(Lactose) = -0.49$
$\sigma^2_{BY}(Lactose) = 0.69$
Lactose
Lactate
chrXIV (481,897) marker
$\bar{x}_{BY}(LiCl) = -0.001$
$\sigma^2_{BY}(LiCl) = 0.86$
$\bar{x}_{BY}(Trehalose) = -0.28$
$\sigma^2_{BY}(Trehalose) = 0.77$
Trehalose
Lithium chloride
B
Number of covariance interactions
Positive
Negative
chrXII (760,027)
chrXII (640,447)
chrXIV (481,897)
chrVIII (100,075)
chrXIV (461,865)
chrXIII (45,801)
chrXV (140,012)
chrXIV (361,531)
chrXV (160,644)
Loci
C
chrXIII (45,801) marker
BY allele
$\bar{x}_{BY}(Paraquat) = -0.2$
$\sigma^2_{BY}(Paraquat) = 0.82$
$\bar{x}_{BY}(Cu) = 0.34$
$\sigma^2_{BY}(Cu) = 0.52$
RM allele
$\bar{x}_{RM}(Paraquat) = 0.2$
$\sigma^2_{RM}(Paraquat) = 1.08$
$\bar{x}_{RM}(Cu) = -0.33$
$\sigma^2_{RM}(Cu) = 1.26$
Normalized growth in Paraquat
Normalized growth in Cu

Figure 4

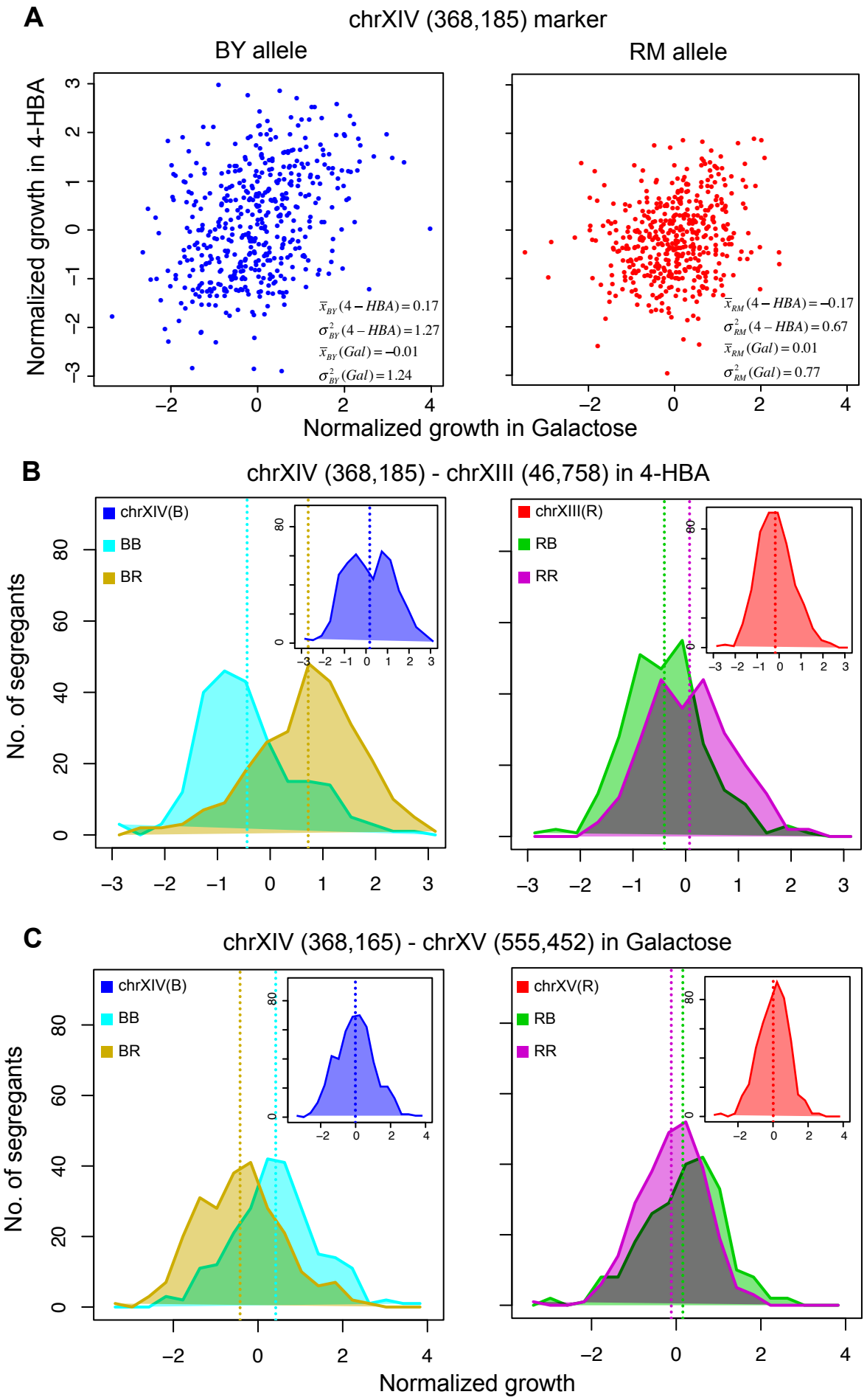

A
chrXIV (368,185) marker
BY allele
RM allele
Normalized growth in 4-HBA
Normalized growth in Galactose
$\bar{x}_{BY}(4-HBA)=0.17$
$\sigma^2_{BY}(4-HBA)=1.27$
$\bar{x}_{BY}(Gal)=-0.01$
$\sigma^2_{BY}(Gal)=1.24$
$\bar{x}_{RM}(4-HBA)=-0.17$
$\sigma^2_{RM}(4-HBA)=0.67$
$\bar{x}_{RM}(Gal)=0.01$
$\sigma^2_{RM}(Gal)=0.77$
B
chrXIV (368,185) - chrXIII (46,758) in 4-HBA
chrXIV(B)
BB
BR
chrXIII(R)
RB
RR
No. of segregants
C
chrXIV (368,165) - chrXV (555,452) in Galactose
chrXIV(B)
BB
BR
chrXV(R)
RB
RR
No. of segregants
Normalized growth

Figure 5

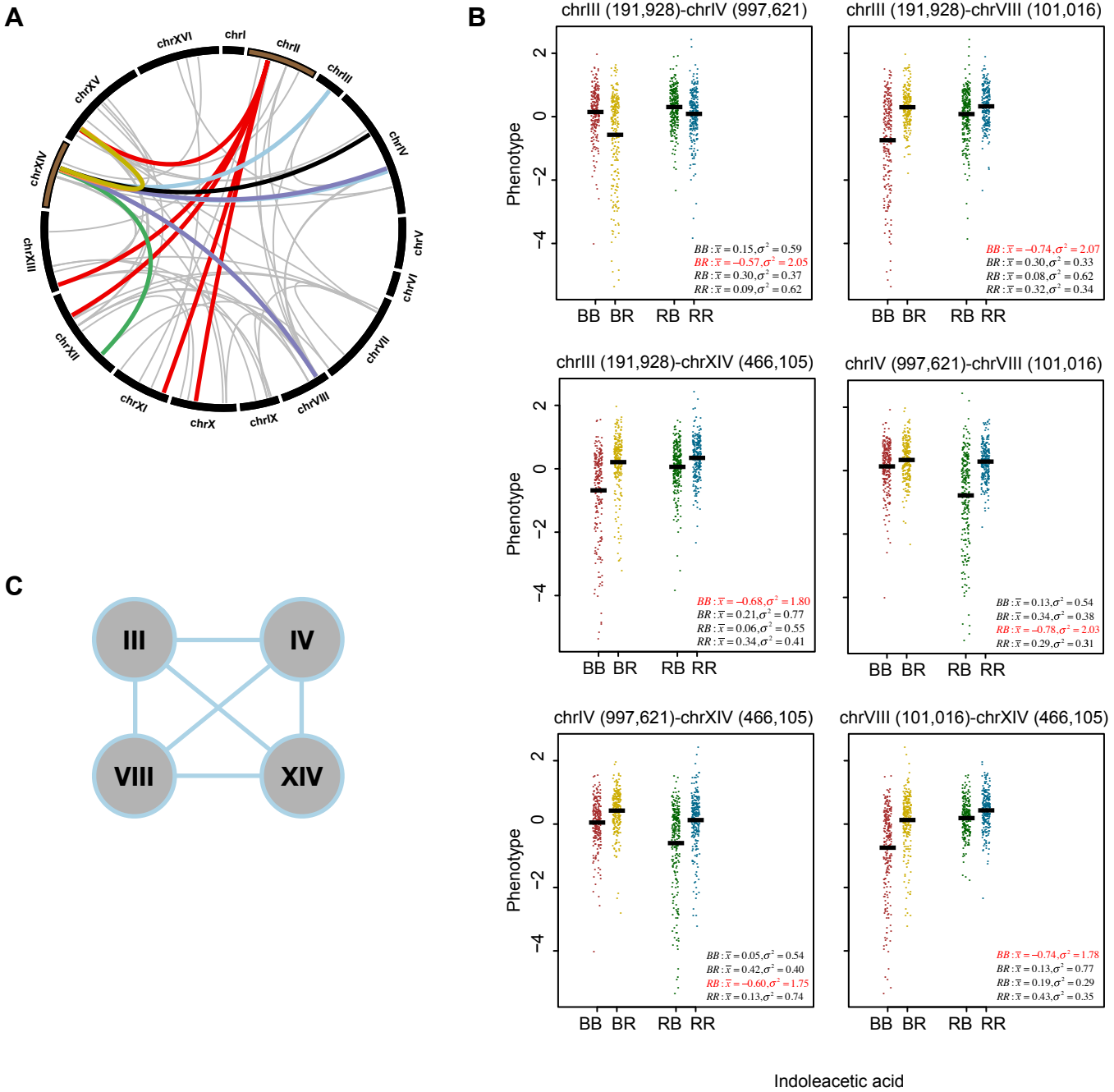
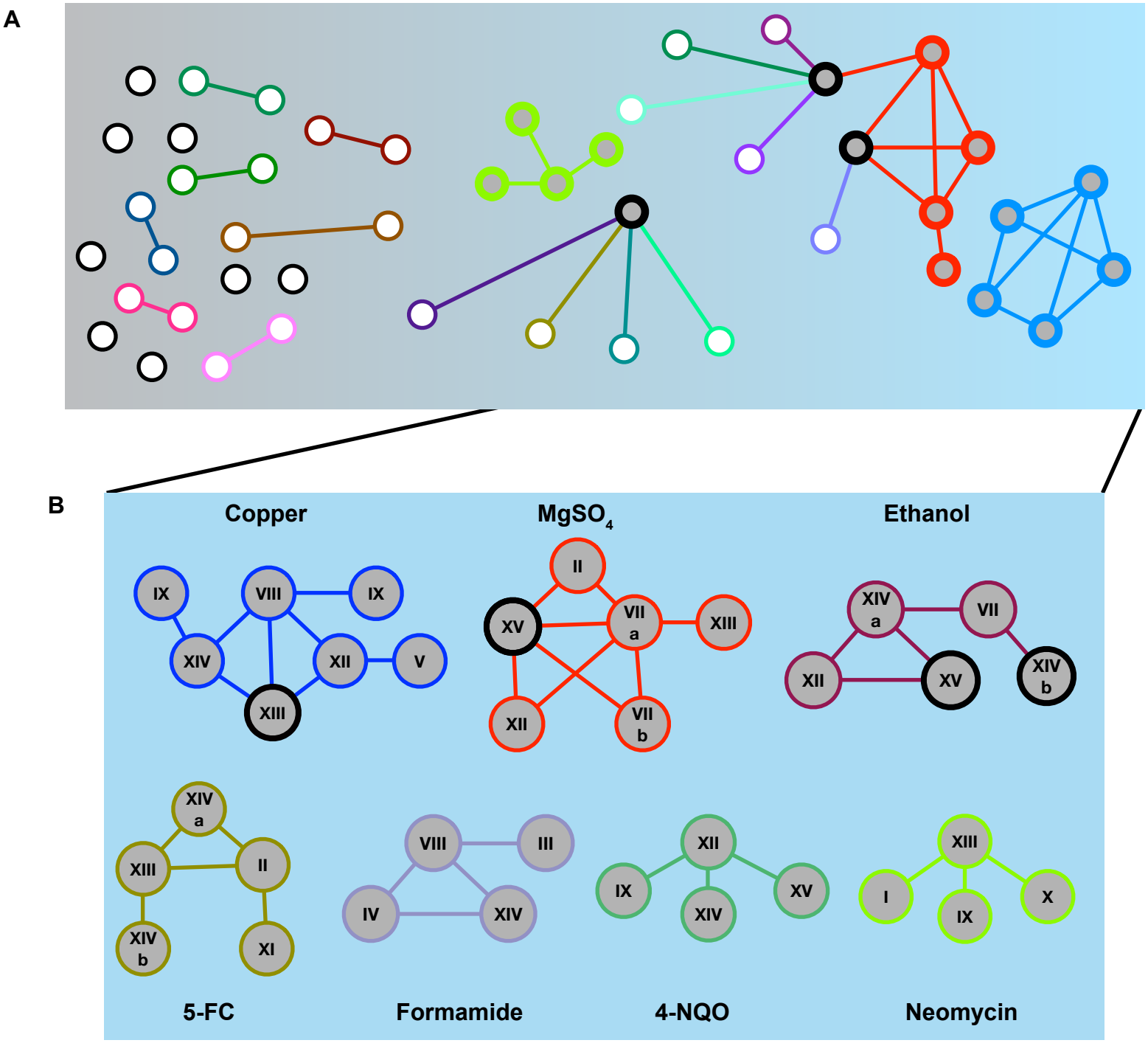

A
chrI
chrII
chrIII
chrIV
chrV
chrVI
chrVII
chrVIII
chrIX
chrX
chrXI
chrXII
chrXIII
chrXIV
chrXV
chrXVI
B
chrIII (191,928)-chrIV (997,621)
chrIII (191,928)-chrVIII (101,016)
chrIII (191,928)-chrXIV (466,105)
chrIV (997,621)-chrVIII (101,016)
chrIV (997,621)-chrXIV (466,105)
chrVIII (101,016)-chrXIV (466,105)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

Figure 6


A
B
Copper
IX
VIII
IX
XIV
XII
V
XIII
MgSO4
II
XV
VII a
XIII
XII
VII b
Ethanol
XIV a
VII
XII
XV
XIV b
XIV a
XIII
II
XIV b
XI
5-FC
VIII
III
IV
XIV
Formamide
XII
IX
XIV
XV
4-NQO
XIII
I
IX
X
Neomycin