

1 Dear recommender,

2 Below are our responses to first round of reviews by PCI Evol Bio. Authors responses are in blue
3 underlined text.

4 Please note that the formatting of the manuscript has changed, as it is being submitted for review
5 to *Genome Biology*'s Special Issue in "Evolutionary Genomics."

6 Thank you,

7 Katie Lotterhos (lead author)

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12 Round #1

13 **Decision**

14 *by Sebastian Ernesto Ramos-Onsins, 2018-01-03 16:00*

15 Manuscript version: <https://doi.org/10.1101/202481>

16 **This preprint merits a revision**

17 This manuscript addresses an important subject regarding the differential effect of genes in
18 diverse environments, their role in local adaptation and the interference between genes that are
19 physical linked but have divergent behaviour in different environments. The authors aim to
20 contrast the hypothesis that "evolution in complex environments should select for modular
21 genetic architectures with limited pleiotropy among modules". The manuscript uses innovative
22 approaches in this field to analyse the interaction between the environmental information of each
23 individual, the genetic linkage of genes and the genetic polymorphisms. The use of
24 co-association networks and galaxy biplots are especially informative and show the main results
25 of this work. Also, it is of crucial importance the simulation analysis performed in this
26 manuscript to understand the possible expected patterns under different scenarios; this analysis
27 includes three different demographic histories (isolation by distance from (i) equilibrium,
28 expansion from (ii) single-refugia or from (iii) two-refugia) and explore the allele frequencies of
29 loci under neutral and under positive selection, contrasted with information on a number of
30 generated environmental variables.

31 The authors used as a model species for such analysis the species *Pinus contorta* (lodgepole
32 pine). Using co-association networks identified several non-overlapping modules of genes

33 associated with environmental factors (Aridity, Freezing, Geography and Multi, this last not
 34 clearly associated to a single environmental factor). Surprisingly, physical linkage was observed
 35 between genes associated with different climate modules but seem not to affect importantly the
 36 different modularity of genes to different environments. The results obtained here using novel
 37 approaches in this field may help to understand the effects of complex and heterogeneous
 38 environments over the genetic variability and over the gene interactions in the genome.

39 Nevertheless, the manuscript needs to be more clearly explained. Although there are many
 40 concepts extensively described in the introduction, it is often difficult to understand the meaning
 41 of sentences including words such as modularity, architecture and pleiotropy, which can have
 42 different meanings in different context. The two reviewers also coincide in the difficulty to
 43 understand some sections of the manuscript.

44 We admit that these terms are difficult to write about because, as noted, they are used in many
 45 different ways in the literature. We have taken the valuable advice of the reviewers and (i)
 46 rewritten the introduction, (ii) added a table to help with terminology, and (iii) reframed and
 47 expanded on major areas of the discussion.

48 I am especially curious about the simulation methods. I believe the methodology used for
 49 simulation, as well as the specific parameters used, should be included with more detail in order
 50 to facilitate the replication of the analysis. I am wondering about the possibility of including the
 51 effect of recombination in such simulations, which may also help to the interpretation of the data.

52 Since these simulations have already been published and code used to reproduce all analyses will
 53 be included with the manuscript, certain details were omitted in the first version. We have
 54 improved the manuscript with more details about this section.

55 Finally, the two reviewers give an essential number of comments that the authors must follow
 56 before having the recommendation of PCI in Evolutionary Biology. Please answer all the
 57 comments of the reviewers separately in a separated text and detail all the modifications included
 58 in the manuscript. I encourage the authors to revise this manuscript following comments from
 59 this round of review and resubmit to PCI in Evol. Biol.

60 We hope you find our revised manuscript worthy of recommendation.

61 Dr. Sebastian E. Ramos-Onsins

62 Reviewer #1

63 *Reviewed by Tanja Pyhäjärvi, 2017-12-05 14:45*

64 Review of Modular environmental pleiotropy of genes involved in local adaptation to climate
 65 despite physical linkage

66 Katie E. Lotterhos, Kathryn Hodgins, Sam Yeaman, Jon Degner, Sally Aitken

67 <https://doi.org/10.1101/202481>

68 The paper presents (to my knowledge) a novel approach to analyse environmental adaptations
 69 using genetic polymorphism and environmental data. The main idea is to inspect the modularity

70 of genetic architecture by joint co-association network analysis of environment and genetic
 71 polymorphism. The authors use experimental data from *Pinus contorta* exome sequencing with
 72 climate data. In addition, they simulate multiple demographic, selective and neutral scenarios to
 73 test the behaviour of the method. The concept and evolutionary implications of multivariate
 74 nature of climatic variation were nicely explained. Also, discussion on the caveats of using the
 75 principal components (or equal approach) to summarize environmental variation's effect on
 76 biological organisms was important to bring up. The new approach is attractive and applicable to
 77 wide set of systems. Clarifying some aspects of the analysis would improve getting the main
 78 message through.

79 Major comments:

80 The preprint presents what I presume to be a reanalysis of exome resequencing genetic
 81 polymorphism data across 281 *Pinus contorta* populations from Yeaman et al. 2016 (Science).
 82 However, the authors should state clearly whether this is the same data or something that has not
 83 been published yet.

84 [This is now more clearly stated in the 2nd to last paragraph of the “Background” section and in](#)
 85 [the “Methods”.](#)

86 Because the modularity is taken into account for both gene groups as well as for environment, it
 87 was a bit confusing at some points which modularity was referred to. First I assumed that the
 88 paper only presents modularity in environment (as suggested by the title). However, the abstract
 89 presents modularity mostly for the genetic architecture. The basic idea of modularity of genetic
 90 architecture could be presented more clearly from the beginning (or in the title?) via an example
 91 or maybe even a figure presenting the both to make it easier to grasp from the beginning. Please
 92 explain clearly what is meant and is there a difference between: “evolutionary modules of loci”,
 93 “selectional modularity of architecture”, “modularity if selectional pleiotropy”,
 94 “developmental/functional modules” and “environmental module” or are they essentially the
 95 same thing.

96 [Thank you for these useful suggestions to improve terminology and accessibility of the](#)
 97 [manuscript - we agree that these concepts are wrought with different meanings in the literature,](#)
 98 [which makes them difficult to write about clearly. To be clear, the co-association network is](#)
 99 [based on modularity of loci in their associations with the environment \(the genetic architecture\),](#)
 100 [and not the modularity of the environment itself. Since this is a new presentation of pleiotropy](#)
 101 [not previously addressed in the literature, we coined the term “environmental pleiotropy” to](#)
 102 [capture what the co-association networks are measuring. To clarify terminology, we have added a](#)
 103 [table \(Table 1\) and a new conceptual figure.](#)

104 Why were SNPs that were already top candidates based on preliminary analysis only used in the
 105 co-association analysis? If the univariate model is likely to miss some signals of adaptation, why
 106 limit only to SNPs based on correlation with single environmental variable?

107 [To answer the first question, the simulations show that neutral loci can form interesting](#)
 108 [co-association networks, and so it is important to exclude them and only use SNPs with high](#)
 109 [confidence for adaptation. The co-association network approach represents a framework for](#)
 110 [interpretation once top candidates are identified, not another genome scan method to search for](#)
 111 [outliers. This is now more clearly stated in the paragraph stated on lines 117.](#)

112 In response to the second question, we do not state in the manuscript that univariate models are
113 likely to miss some signals of adaptation. We do state that associations with principal
114 components may miss some signals of adaptation that are detected with univariate associations.
115 Univariate methods are probably going to miss adaptation loci, but that question was not what
116 we were interested in addressing with this analysis. The goal isn't to identify new loci, but rather
117 to use clustering to better interpret how selection has shaped the variation.

118 The authors state that the results are not sensitive to the distance threshold used in the clustering
119 of the networks. What other thresholds were tested and why 0.1 was chosen as a threshold?

120 We chose 0.1 as a threshold based on the simulations. The simulated data showed that this
121 threshold enriched for selected loci to be strongly connected to each other in a co-association
122 network, and for a lack of connections among neutral loci with each other or among neutral and
123 selected loci. This is now shown more clearly in a new figure (Supplementary Figure 13).

124 While we recognize that it may be advantageous for all studies to use the same threshold, at the
125 same time we do not want to advocate that this threshold be used for all studies because there
126 may be some nuances for genomes from different species.

127 Further, as the co-association analysis is in the core of the paper, it would be interesting to know,
128 why the authors ended up using the division into 4 clusters. Are the conclusions dependent on
129 the number of clusters?

130 To be clear, this division into four main clusters is for the purposes of presentation only, and was
131 necessary (i) for clear data visualization and (ii) computational limits. This is now more clearly
132 stated in the “Environmental response modules” section of “Results”. We believe that each
133 individual module - as visualized with the co-association network - are more meaningful
134 representation of groups of loci that respond similarly to the environment.

135 To emphasize this better in the revised manuscript, we re-colored and re-ordered loci in Figure 1
136 (and subsequent figures) according to their co-association module (rather than by the number of
137 outlier SNPs in the first version). We hope you will find this new visualization a better
138 representation of the data, as we have.

139 For example, the distance between “Freezing” and “Geography” is not very large and the clusters
140 are actually quite similar based on visual inspection.

141 The “Geography” group differs from the “Freezing” group in two important ways that are shown
142 in the clustering/network plot and galaxy plots. Most Geography SNPs have associations with
143 latitude but not frost variables, while most Freezing SNPs have associations with frost variables
144 but not latitude.

145 Also, will the clustering be identical when structure corrected associations are used?

146 Interestingly, the clustering is not perfectly identical with the structure-corrected associations
147 were used, although it results in broadly similar associations as shown in the Supp Mat. We
148 argue that raw associations would be the correct associations to use, because sometimes

149 [correction for structure changes the sign of the association because of the orientation of the SNP](#)
 150 [frequency relative to the neutral structure in multivariate space \(as can be visualized by](#)
 151 [comparing the raw and corrected patterns the galaxy plots\).](#)

152 Minor comments:

153 Simulations were a great addition. Please give more details about the demographic simulations.
 154 For example, what was the strength and duration of selection in relation to demographic history
 155 in the simulations?

156 [We added these details to the methods.](#)

157 Exactly how many individuals were sampled per population? Is each of the 281 populations
 158 represented by single individual? If only one per population was sampled, how were the allele
 159 frequencies for Bayenv2 obtained? Do seedlots refer to a set of seeds from multiple trees, e.g.
 160 can it be assumed to be a random sample from the population?

161 [We added these details to the methods and to the last two paragraphs of the “Background”](#)
 162 [section. Basically, a “seedlot” is a sampling location.](#)

163 How does your approach relate to the idea of analysing gene networks jointly when identifying
 164 the genetic basis of local adaptation that was presented e.g. by Daub et al. 2013?

165 [We assumed the reviewer was referring to the paper. “Evidence for Polygenic Adaptation to](#)
 166 [Pathogens in the Human Genome.” In this paper they use a gene set enrichment approach, in](#)
 167 [which *a priori* sets of specific biological pathways are tested for enrichments of associations](#)
 168 [with phenotypes.](#)

169 [Our approach is not a gene set enrichment approach, and we would not be able to identify](#)
 170 [specific biological pathways in our data because many of our top candidates are not annotated. In](#)
 171 [addition, co-association networks are not an enrichment approach, but a framework for](#)
 172 [understanding multivariate patterns of associations across candidate genes. We did test for](#)
 173 [enrichment of GO terms in our candidate set, and these are presented in the results and](#)
 174 [supplementary tables.](#)

175 [Our approach is more similar to the use of co-expression networks used to identify modules in](#)
 176 [RNAseq data. Our approach is distinct from the application of bipartite networks previously](#)
 177 [applied to the study of pleiotropy, and this is now discussed in the section “Benefits and caveats](#)
 178 [of co-association networks” in the Discussion.](#)

179 Add citation to Hill and Robertson 1966

180 [This paper is cited in the “Background” section.](#)

181 Structure correction is mentioned briefly, but what is the overall structure pattern in *P. contorta*?

182 [The overall structure pattern is one of isolation by distance, which is now indicated in the 2nd to](#)
 183 [last paragraph of “Background”.](#)

184 “Across” repeated in Linkage disequilibrium part.

185 [Fixed.](#)

186 What do you mean by “SNPs from most genes associated with only a single climate module”?
 187 Based on figure 1, each SNP can only be associated with one network.

188 [This sentence was rewritten for clarify.](#)

189 Could you present what kind of results would reject the Hypothesis of Modular Pleiotropy?

190 [We now use Figure 1 to compare the patterns expected under modular vs. universal pleiotropy.](#)

191 Replace “Thus, it..”, with “Thus, it is...” in Discussion.

192 [This sentence has been revised.](#)

193 How sensitive the method is to the choice of environmental variables or SNPs? Are the networks

194 and modules highly dependent on single variables? Also, how many nuisance variables

195 (environmental variation that are not selectively relevant) the analysis tolerates?

196 [From the simulations we performed, we can at least say that co-association networks correctly](#)

197 [cluster selected loci when many variables are measured, but the true causal environment is](#)

198 [excluded \(see “Results: Simulations”\). Thus, the approach appears to be able to tolerate nuisance](#)

199 [variables \(see also “Discussion: Benefits and caveats of co-Association networks”\), although we](#)

200 [did not quantify “how many”.](#)

201 [A fuller evaluation is beyond the scope of this manuscript, as they require a set of more complex](#)

202 [simulations that the lead author has been working on for some time. So, a more comprehensive](#)

203 [evaluation on more realistic simulations is forthcoming.](#)

204 Figure 1a, text in the gray background is tiny and hard to read.

205 [We removed this text from the figure and added a table \(Table 2\) to explain the abbreviations.](#)

206 Figure 3, the shading of the quadrant does not reproduce in the printouts.

207 [Thank you for letting us know. The shading of the quadrants did reproduce on our printers, but](#)

208 [we darkened the shading slightly for the revised manuscript.](#)

209 Figure 2, The among-group LD patterns are almost invisible in the screen and completely

210 invisible in the print-out.

211 [Note that the LD data had to be transformed to be plotted with the recombination rates, and this](#)

212 [transformation affected some of the visualization. Generally, this made patterns of LD less than](#)

213 [0.05 less visible, but these are negligible. We also provide a supplementary figure of LD](#)

214 [patterns.](#)

215 Tanja Pyhäjärvi, University of Oulu, Finland

216 [Thank you so much for your useful comments!](#)

217

Reviewer #2

218 SUMMARY of Modular environmental pleiotropy of genes involved in local adaptation to

219 climate despite physical linkage

220 The authors present an important advance in how we think about and measure pleiotropy using
221 co-association networks and galaxy plots. As a geneticist studying pleiotropy and someone who
222 is deeply interested in the genotype to phenotype map, I am very intrigued by these results and
223 novel methods. The figures are beautiful and well thought out. The section in the introduction
224 about the debate surrounding how to define traits (cold, dry, cold-dry) is among the clearer parts
225 of the paper. A major impact of the paper is the ‘co-association network’ approach, which solves
226 the aforementioned debate by finding clusters of genes that define relevant traits (or in this case,
227 relevant environmental responses).

228 A major problem with the paper is that it is often unclear and important sections are difficult to
229 follow or seemingly left out. Because the authors are studying pleiotropy, they necessarily have
230 to deal with multiple complicated topics at once, including (in their words), “the modularity of
231 the architecture (number of distinct climate factors), overlap among modules, and physical
232 linkage among loci”. There are many times when I am not sure which of these topics is being
233 addressed. Some of this confusion comes from terminology, for example, genetic architecture
234 usually refers to genome structure or linkage, but occasionally the authors also use architecture
235 to refer to the number of distinct climate factors. The word modularity can also be very tricky
236 because it can refer to the number of distinct climate modules or the number of distinct genetic
237 modules that respond to these climates. The terminology in this manuscript should be used more
238 carefully.

239 [We agree that it is difficult to write about these terms which are used in many different ways in
240 the literature. In this new version of the manuscript we have carefully edited for terminology and
241 semantics, including a new introduction, table for terminology, and conceptual figure.](#)

242 The first sentence of the abstract is another good example of the unclearness: “Physical
243 proximity among alleles shaped by different sources of selection is a fundamental aspect of
244 genetic architectures critical for predicting their evolution”. I did not understand this sentence at
245 all. In addition to terminology concerns (e.g. genetic architecture is not yet defined), there are
246 major grammatical errors that prevent a reader from discerning the object and subject of the
247 sentence (e.g. are the allele frequencies shaped by selection, or is their proximity shaped by
248 selection? which of these are you trying to predict?).

249 [This sentence refers to physical linkage among genes and has been revised for clarity. Both allele
250 frequencies and the proximity of alleles may be shaped by selection, and in this manuscript we
251 are not trying to predict either but rather describe them in a way for understanding pleiotropy and
252 modularity.](#)

253 Further, this sentence summarizes many different, difficult concepts simultaneously and it is
254 unclear which is the main focus. It would be better to start with one central concept, and then
255 step through the next concepts one at a time. i.e. “Are there modules of genes that respond to
256 particular environmental changes? This is important to understand for reasons X, Y, and Z. We
257 might be able to answer this question by looking at [describe data]. But physical linkage among
258 loci can obscure this signal...”

259 [The entire introduction has been revised and reorganized to emphasize the main focus of the](#)
 260 [study. We have also added a conceptual figure to explain the framework and guide the reader.](#)

261 Given this paper has the potential to demonstrate high impact findings, it is worth the time to
 262 restructure the manuscript, stepping through each topic more slowly and making clear to the
 263 reader (perhaps with numbered lists in text) what are the difficulties in measuring the extent of
 264 pleiotropy and how this method deals with those difficulties. Sometimes, instead of relying on
 265 words like “pleiotropy,” perhaps it would improve clarity to talk about “genetic changes that
 266 affect multiple environments”, and the genotype- phenotype map. Instead of staying so close to
 267 the terminology, and to the literature regarding modular pleiotropy, I wonder if the authors might
 268 use less technical terms to explain the motivation for this work, and its significance to a broader
 269 audience.

270 [We appreciate that you highlight the potential for high impact of this research. We want ~~it~~ this](#)
 271 [study to reach a broad audience, while at the same time guiding the reader through the different](#)
 272 [ways these terms have been used in the literature and how terms relate to our study. In this new](#)
 273 [version we added a Table for terminology, and taken care to use less technical terms in the](#)
 274 [introduction.](#)

275 In sum, this manuscript presents a novel approach to studying the genotype-phenotype map, and
 276 also a novel way of thinking about and defining phenotype. I recommend the authors re-structure
 277 the paper, stepping through its important contributions one at a time to improve clarity.

278 INTRODUCTION

279 This sentence in the introduction indicates an omission of the literature: “Although there is
 280 emerging agreement that organisms have modular organization of genes in their effects on
 281 phenotypes.” There does not seem to be agreement at all and I think the authors should
 282 acknowledge this. The cited paper from Boyle et al 2017 did not acknowledge this and authors
 283 apologized over Twitter. There are many papers by Hill (1,2) and by Rockman (3) that do not
 284 support the modular pleiotropy model. Further, these are many “reply to” papers that highlight
 285 just how controversial this topic remains (1,4,5).

286 The introduction is a little bit unfocused. The authors review a great deal of literature and
 287 terminology, but the main message of the paper is a little lost. I think this is a difficult task
 288 because so many issues are tackled in this introduction. A single sentence early on explaining a
 289 single, simple important contribution might help a reader focus.

290 [The introduction has been completely revised, with the intent to provide a roadmap for the](#)
 291 [reader.](#)

292 There seems to have been another study that generated some important data used in the current
 293 study. I don't understand, even after re-reading the paragraph many times, what these data look
 294 like. This seems problematic, as these data are key to the current study. What are “candidates”?
 295 Are these “candidate loci” or “candidate trees”? How many are there?

296 [In this revision, we have taken care throughout to specify whether we are talking about](#)
297 [“candidate SNPs” or “candidate genes.”](#)

298 Why is convergent adaptation relevant here? This paragraph should start off with a comment
299 about pleiotropy, and how these data will allow authors to study pleiotropy. Not all details from
300 this dataset need to be described, but the details relevant to the insights about modularity and
301 pleiotropy should be described here. How do these data differ from those used in previous
302 studies of pleiotropy? Does this feature of the data give you more power?

303 [Because this is a re-analysis of the same dataset, it is important to describe how this approach is](#)
304 [yielding insight not achieved in the previous publication where convergent adaptation was the](#)
305 [focus. We added some of the details requested by the reviewer to show the uniqueness of our](#)
306 [dataset in the last two paragraphs of the “Background”.](#)

307 Authors sometimes omit the word “frequencies” from the phrase “allele frequencies”. Authors
308 should be clearer when they are talking about an allele itself versus its measured frequency
309 across environments.

310 [We found two instances where the word “frequency” was omitted for this phrase and these were](#)
311 [revised.](#)

312 How does a co-association network analysis “characterize pleiotropy and linkage of the
313 architecture?” What features does it use? Is this a new approach? What are its benefits? What
314 novel insights might it provide? I understand that this is the introduction, so you don’t want to
315 give away the results. But you must state something about this novel approach and why it is a
316 good idea.

317 [We addressed these points through a new conceptual figure, now Figure 1.](#)

318 METHODS:

319 This is very difficult for anyone to read who does not study plants.

320 What is a seedlot?

321 How do you have needle tissue? I thought you only have seeds (“seed”lot)

322 Did you grow these trees in different environments? Or did you sample trees from different
323 environments and calculate how allele frequency changes across environments? This part of the
324 methods is really unclear.

325 A seedlot can be thought of as a sampling location. Seeds were collected from different sampling
326 locations, grown in a common garden, and needle tissues were collected and sequenced. This is
327 now clearer in the “Methods” section.

328 Why did you obtain samples and extract sequence information if Yeaman et al 2016 already did
329 this. Do you have different samples? What is the difference between your work and the Yeaman
330 study?

331 The same dataset was used, but the Yeaman 2016 focused only on comparative genomics and
332 convergent adaptation with another species. This is now clearer in the last two paragraphs of
333 “Background”. We added additional clauses to the methods to highlight which analyses were the
334 same as those already reported.

335 The co-expressed genes section is also missing relevant details. What 7 climates? How many
336 plants per cluster? How did you choose the plants in each cluster?

337 The co-expression data was previously published, so some relevant details were glossed over in
338 the first draft. We have now included more details and clarified our analysis with respect to the
339 gene expression patterns. In the gene expression analysis, the individual plants were not
340 clustered, but rather genes were clustered based on their similarity in expression profile across
341 the seven climate treatments tested in the earlier paper.

342 RESULTS:

343 In the first paragraph, again it is not very clear what is relevant about these top candidates in
344 terms of pleiotropy or modularity. Why are you studying them? You provide information about
345 how many there are before really explaining why they are in the paper.

346 The study of pleiotropy and modularity is relevant only to loci under selection and not neutral
347 loci, which are putatively the top candidate genes and the top candidate SNPs within the genes.
348 We added text in “Background” and a sentence at the beginning of the “Results” to clarify this.

349 In paragraph 2, again the motivation is not clear. The co-association method is described nicely
350 in the methods. But I am left to infer what these SNP-environment associations mean and why
351 you might want to cluster them. At the end of the co-association network section, perhaps add a
352 final sentence to summarize the observation. Which of your hypotheses was supported and what
353 does this mean about selection and about pleiotropy?

354 [We hope that you find the new conceptual figure helpful in providing the motivation for this](#)
 355 [section and summarizing the hypotheses. Much of the “Discussion” has been expanded to](#)
 356 [address these questions in further detail.](#)

357 I think the section on PCA is appropriate to include as it finally explains some of the benefits of
 358 co-association networks over other approaches.

359 The section on benefits and caveats of co-association networks also seems beneficial to include.
 360 However, it focuses on yet another impact of this study, identifying high confidence SNPs. It
 361 does not focus much on pleiotropy or modularity, these words are not even used. It would be
 362 very beneficial to pick a single contribution of this paper and continually come back to it in the
 363 text. It is not necessarily confusing to include multiple contributions and insights within a single
 364 manuscript, but one in particular should be highlighted as the main focus. Otherwise the paper
 365 reads as unfocused and the motivation/impact is unclear.

366 [The entire “Discussion” has been revised and expanded to illustrate the three main insights](#)
 367 [gained by using the approach \(sections of “Discussion” starting with “Genetic architecture of](#)
 368 [adaptation...”.](#) We hope this clarifies the motivation and impact of the study.

369 [The last two sections of the “Discussion” focus on the benefits and caveats of the approach,](#)
 370 [which have been revised to relate more directly to the interpretation of the main insights.](#)

371 In sum, the co-association analysis is a novel and important idea. I hope with some re-
 372 structuring and decisions about how to focus the manuscript this idea will reach a broader
 373 audience.

374 [Thank you for the useful suggestions!](#)

375 1. Hill WG, Zhang X-S. Assessing pleiotropy and its evolutionary consequences: pleiotropy
 376 is not necessarily limited, nor need it hinder the evolution of complexity. Nat Rev Genet.
 377 Nature Publishing Group; 2012 Apr 1;13(4):296–6.

378 2. Hill WG, Zhang X-S. On the Pleiotropic Structure of the Genotype–Phenotype Map and
 379 the Evolvability of Complex Organisms. Genetics. Genetics; 2012 Mar 1;190(3):1131–7.

380 1. Rockman MV. The QTN program and the alleles that matter for evolution: all that's gold
 381 does not glitter. Evolution. 2012 Jan;66(1):1–17.

382 2. Paaby AB, Rockman MV. Pleiotropy: what do you mean? Reply to Zhang and Wagner.
 383 Trends Genet. Elsevier; 2013 Jul;29(7):384.

384 3. Wagner GP, Zhang J. Universal pleiotropy is not a valid null hypothesis: reply to Hill and
 385 Zhang. Nat Rev Genet. Nature Publishing Group; 2012 Apr 1;13(4):296–6.