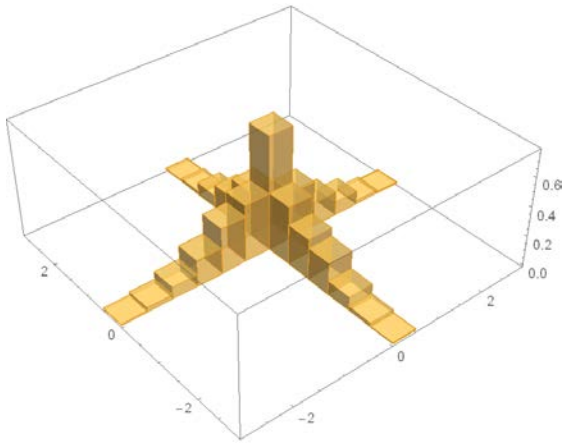
In this manuscript, the authors use individual-based simulations to explore to what extent closely linked loci affecting two separate traits differ from a single pleiotropic locus that jointly affects these traits, in terms of (i) the genetic correlation that is maintained between these traits at a balance between mutation and stabilizing selection (+drift), and (ii) the likelihood to correctly infer the genetic basis of quantitative traits in a GWAS. This study is partly motivated by verbal claims that fully linked loci should be equivalent to a single pleiotropic locus, since they cannot evolve independently from one another. The authors investigate this hypothesis in some detail, from the perspectives of both theoretical evolutionary biology and statistical genetics. I was quite interested to read this manuscript, which addresses a simple fundamental question that seems to not have received a fully satisfying answer yet.

The bulk of the paper focuses on the maintenance of genetic correlations between traits (with 7 out of 8 figures), for which the simulation results are compared to a prediction from Lande (1984) for fully linked loci as a reference. The authors perform an exhaustive analysis, investigating the effect of several key parameters: selection strength and amount of correlative selection, mutation rate and phenotypic effects, linkage, and migration. My main comment would be that, even though it is of course important to assess the influence of all parameters, it is also easy for the reader to get lost, especially since the presentation is mostly descriptive, and some explanation would seem to be required at multiple points. Below is a list of suggestions:

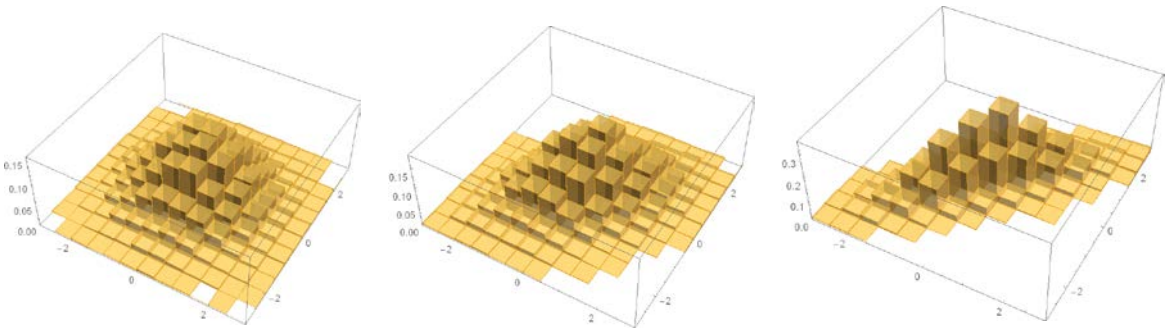
1 - First, I think the authors should explain a bit better what makes a difference between two fully linked loci and a single pleiotropic locus. They have identified an important difference that concerns mutation rates and effects, but I don't find their explanation sufficient. For instance, they write in the conclusion: "Without high mutation rates, the ability to create genetic covariance between linked loci is highly diminished because the combined likelihood of mutations in each linked loci with both mutational effects in the same direction is low." (466-469). I think this argument should be made more explicit earlier in the paper, as it's central to the process the authors are investigating here. The joint distribution of mutations effects on two traits that can mutate via two (fully linked) loci is

$$f(x_1, x_2) = \frac{u_1(1-u_2)f_1(x_1)\delta(x_2) + u_2(1-u_1)f_2(x_2)\delta(x_1) + u_1u_2f_1(x_1)f_2(x_2)}{1-(1-u_1)(1-u_2)}$$

where u_i is the mutation rate at locus i, $f_i(x_i)$ the distribution of mutation effects on trait i at locus i, and $\delta(x)$ is a Dirac Delta function, which equals 0 except at x, and integrates to 1. For Gaussian distributions of mutation effects as here, $f_1(x_1)f_2(x_2)$ in the last term is a bivariate Gaussian with no correlation, similar to the effect of a pleiotropic locus without mutational correlation (as modeled here). However, this last term rapidly vanishes with small mutation rates, such that we're only left with a "cross" distribution, where all the probability mass is on mutational variation on one trait with the other trait fixed at 0. A Dirac delta is not convenient to plot as it goes to infinity, but here's how this looks on simulated draws with $u = u_1 = u_2 = 0.001$:



So the point is really that there is little opportunity for joint change in both traits, which make it difficult to select for genetic correlation. In comparison, the difference between correlated vs uncorrelated mutation effects at a pleiotropic is very mild, as illustrated below (with mutational correlation = 0, 0.5 and 0.9):



2- Another point about mutation is that, as the mutation rate decreases, the Kimura-Lande Gaussian approximation used here is expected to be rapidly replaced by Gillespie's house of cards approximation. In fact, this HoC approximation seems to work fine over all the parameter range explored here, judging by figure S3. So it would seem useful to also report predictions for the genetic correlation based on this approximation, if they exist (perhaps from the Gillespie 1985 multivariate paper cited in the ms).

3 – Regarding the influence of migration, I also think more can be said, in particular about the fact that its "effect on genetic correlation is still observed when there is no correlational selection on the traits in the source population" (272-274). This occurs because of the difference in mean phenotypes between the two populations, coming from the fact they are selected for their different optima. The phenotype distribution in the focal patch is a mixture between the distributions of residents and migrants (and their subsequent crosses: F1, backcrosses, etc). From the law of the total covariance, the covariance of a mixture includes a component caused by differences in means of the underlying distributions, even when their covariances themselves are identical. Here, neglecting the contribution of later generations of crosses between residents and migrants, the local covariance matrix should be inflated by

$$m(1-m)(\bar{\mathbf{z}}_r - \bar{\mathbf{z}}_m)(\bar{\mathbf{z}}_r - \bar{\mathbf{z}}_m)^{\mathrm{T}},$$

with $\bar{\mathbf{z}}_r$ and $\bar{\mathbf{z}}_m$ the mean (bivariate) phenotypes of residents and migrants, respectively. The direction of the genetic correlation produced by this effect depends on the direction of phenotypic divergence between residents and migrants for the two traits. In fact, under strong differentiation this term may have a stronger effect on the genetic correlation than the genetic correlation in migrants themselves (here caused by correlational selection in their population of origin). This is worth discussing and exploring a bit further in your context of linked vs pleiotropic genes.

4 – I have trouble grasping at the main result with respect to GWAS. Here there were no neutral markers in the simulations, so all loci were truly causal (although they may be causal only with respect to one of the traits). This means that the recombination rate between adjacent loci has two effects: it influences (i) the evolution of genetic correlation between traits, and (ii) the association between a non-casual locus and a causal one (with respect to a given trait). This should be written more explicit in the paper, as it bears on the interpretation of the results. From Table 1 and Figure S1, the main result seems to be that, for non-pleiotropic genes, the rate of false positives increases in proportion to the genetic correlation between traits, and for a given genetic correlation depends to a lesser extent on the recombination rate. This makes sense, since genetic correlations comes entirely from LD in this case (the total genetic covariance is just the sum of LD multiplied by allelic effects at pairs of loci), and LD is also what causes association between a trait and a non-causal locus. So by setting the level of genetic correlation, the authors indirectly control the mean LD between adjacent loci (fig S2), which is also the mean LD between a causal and non-local locus for a given trait. It seems that this argument could be made a bit more explicit in the paper, making use of well-known mathematical formulas. You may also want to simulate/discuss what would be the difference with a purely neutral marker. My guess is that the probability of false positives would then mostly depend on recombination distance, because only this will influence LD in this case, not the genetic correlation between traits.

Minor comments:

Figures 1-7: why not also plot the expected minimum correlation under no linkage in these graphs, since this was also predicted by Lande (1984)?

Table 1: it is unclear at first what distinguishes the first 4 lines from the 4 lines below; the difference is the genetic correlation between traits (modified by tuning correlational selection), but this should appear somewhere in the legends or table captions, rather than just in the methods.

3 : “traits do not act independently of one another” is not really clear: traits don’t necessarily “act” on anything. I would just write that traits are not independent, or do not to vary independently.

5-6: “reaching their respective optimal potentials” reads awkward. What’s the optimal potential for a trait? Do you mean the optimum trait value favored by natural selection? If so just write this instead.

61-63: “recombination can break up associations between alleles at linked loci, reducing genetic correlations between the traits they affect, but the same cannot occur with a pleiotropic locus”. Well, in the longer run, duplication followed by sub- or neo-functionalization can alleviate potential costs of pleiotropy. This is worth mentioning somewhere, as it has been discussed as a mechanism leading to the evolution of modularity starting from pleiotropic genes (eg Wagner et al 2007 Nature Reviews Genetics)

74-76: “The detriment of pleiotropic effects is exacerbated when increasing the strength of selection or the correlational selection between traits”. However, increasing correlational selection can rotate the fitness surface, in effect favoring pleiotropic effects causing joint change in multiple traits. How can you reconcile these two apparently contradictory statements?

98-101: “From these equations we see that […] genetic correlation […] depends on […] the mutational inputs (mutation rates and mutational variances)”. This sentence is wrong as stated, since the equation just above (eq. 3) precisely shows that the genetic correlation does not depend on these mutational properties, but only on correlational selection rho. That mutational properties may actually matter is because assumptions leading to eq. (3) don’t always hold, which you explore here, but this is another issue.

175: I imagine that rho_omega is the factor that multiplies omega^2 two lines above, but please make this explicit.

182-183: This is another clear difference between pleiotropy and linkage: fully linked non-pleotropic loci have uncorrelated effects on the two traits, while pleiotropic genes may have correlated effects (even though here you assume a covariance of 0).

242-244: “A decrease in correlational selection […] has a larger effect on maintaining strong genetic correlations than a decrease in selection strength”. This sentence seems wrong as stated. Why not just write that with the parameters you chose, a decrease in correlational selection reduces genetic correlation more than does a decrease in the strength of selection? (your sentence seems to mean the reverse, ie that higher genetic correlation is maintained under reduced correlational selection). Note however that these are difficult to compare, since they concern different parameters, which are expected to have different influences on on genetic correlations anyway based on analytical predictions, eg your eq. (3).

248-249: Perhaps you should refer here to figure S3 to convince the reader that that the equilibrium has been reached after 10000 generations at mutation rates below 10^-3. Note also that the mutation rate is not provided in figs 3,4, I imagine it it also 10^-3 as in fig 2?

347-350: what you describe here is the Bulmer effect (Bulmer 1971), whereby negative LD reduces genetic variance under stabilizing selection. However, it's a bit unclear how you here extend this to the correlation between traits. Note also that this build-up of negative LD also occurs in the model by Lande (1976 for a single trait, and 1980, 1984 for multiple traits), so it does not seem to really distinguish strong from weak selection.