

# Interpreting population and family-based genome-wide association studies in the presence of confounding

Carl Veller and Graham Coop

Department of Evolution and Ecology, and Center for Population Biology,  
University of California, Davis, CA 95616

## Abstract

A central aim of genome-wide association studies (GWASs) is to estimate direct genetic effects: the causal effects on an individual's phenotype of the alleles that they carry. However, estimates of direct effects can be subject to genetic and environmental confounding, and can also absorb the 'indirect' genetic effects of relatives' genotypes. Recently, an important development in controlling for these confounds has been the use of within-family GWASs, which, because of the randomness of Mendelian segregation within pedigrees, are often interpreted as producing unbiased estimates of direct effects. Here, we present a general theoretical analysis of the influence of confounding in standard population-based and within-family GWASs. We show that, contrary to common interpretation, family-based estimates of direct effects can be biased by genetic confounding. In humans, such biases will often be small per-locus, but can be compounded when effect size estimates are used in polygenic scores. We illustrate the influence of genetic confounding on population- and family-based estimates of direct effects using models of assortative mating, population stratification, and stabilizing selection on GWAS traits. We further show how family-based estimates of indirect genetic effects, based on comparisons of parentally transmitted and untransmitted alleles, can suffer substantial genetic confounding. In addition to known biases that can arise in family-based GWASs when interactions between family members are ignored, we show that biases can also arise from gene-by-environment ( $G \times E$ ) interactions when parental genotypes are not distributed identically across interacting environmental and genetic backgrounds. We conclude that, while family-based studies have placed GWAS estimation on a more rigorous footing, they carry subtle issues of interpretation that arise from confounding and interactions.

## 1 Introduction

Genome-wide association studies (GWASs) have identified thousands of genetic variants that are associated with a wide variety of traits in humans. In the standard ‘population-based’ approach, the GWAS is conducted on a set of ‘unrelated’ individuals. The associations that are detected can arise when a variant causally affects the trait or when it is in tight physical linkage with causal variants nearby.

Central to the aims of GWASs is the estimation of variants’ effect sizes on traits of interest. These effect size estimates are important for identifying and prioritizing variants and implicated genes for functional followup, and may be used to form statistical predictors of trait values or to understand the causal or mechanistic role of genetic variation in traits. Understanding sources of error and bias in GWAS effect size estimates is therefore crucial.

The interpretation of GWAS effect size estimates is complicated by four broad factors (Vilhjálmsson and Nordborg 2013; Young et al. 2019). First, the causal pathways from an allele to phenotypic variation need not reside in the individuals who enrolled in the GWAS, but can also reflect causal effects on the individual’s environment of the genotypes of their siblings, parents, other ancestors, and neighbors (indirect genetic effects, or dynastic effects; Wolf et al. 1998). Second, a phenotypic association can result from correlations between the allele and environmental causes of trait variation (environmental confounding; Lander and Schork 1994). Third, a phenotypic association can be generated at a locus if it is genetically correlated with causal loci outside of its immediate genomic region (genetic confounding; Vilhjálmsson and Nordborg 2013). Fourth, an allele’s effect on a trait might depend on the environment and the allele’s genetic background (gene-by-environment and gene-by-gene interactions, or  $G \times E$  and  $G \times G$ ; Freeman 1973; Marchini et al. 2005; Gauderman et al. 2017).

Since our primary interest here will be genetic confounding, we briefly describe some potential sources of the long-range allelic associations that drive it: population structure, assortative mating, and selection on the GWAS trait.

Population structure leads to genetic correlations across the genome when allele frequencies differ across populations or geographic regions: sampled individuals from particular populations are likely to carry, across their genomes, alleles that are common in those populations, which induces correlations among these alleles, potentially across large genomic distances. Such genetic correlations persist even after the populations mix, as alleles that were more common in a particular source population retain their association until uncoupled by recombination.

Assortative mating brings alleles with the same directional effect on a trait (or on multiple traits, in the case of cross-trait assortative mating) together in mates, and therefore bundles these alleles in offspring and subsequent generations. This bundling manifests as positive genetic correlations among alleles with the same directional effect (Wright 1921; Crow and Felsenstein 1968), which can confound effect size estimates in a GWAS on the trait.

Finally, natural selection on a GWAS trait can result in genetic correlations by favoring certain combinations of trait-increasing and trait-decreasing alleles. A form of selection that is expected to be common for many traits of interest is stabilizing selection, which penalizes deviations from an optimal trait value. By favoring compensating combinations of trait-increasing and trait-decreasing alleles, stabilizing selection generates negative correlations among alleles with the same directional effect (Bulmer 1971, 1974), and therefore can confound effect size estimates in a GWAS performed on the trait under selection or on a genetically correlated trait.

The potential for dynastic, environmental, and genetic confounds to bias GWAS effect size estimates has long been recognized (Lander and Schork 1994; Ewens and Spielman 1995), and so a major focus of the literature has been to develop methods to control for these confounds (Pritchard and Rosenberg

46 1999; Price et al. 2010). Standard approaches include using estimates of genetic relatedness as covariates  
47 in GWAS regressions (Price et al. 2006; Yang et al. 2014) or downstream analyses such as LD-Score  
48 regression (Bulik-Sullivan et al. 2015a,b; Bulik-Sullivan 2015). Such methods aim to control for both  
49 environmental and genetic confounding, but do so imperfectly (e.g., Berg et al. 2019; Sohail et al. 2019).  
50 Further, it is often unclear what features of genetic stratification are being addressed (Vilhjálmsson and  
51 Nordborg 2013; Young et al. 2019): assortative mating in particular may not be well accounted for by  
52 these methods (Border et al. 2022b). Moreover, in reality, there is no bright line separating dynastic,  
53 environmental, and genetic confounding.

54 One promising way forward is to estimate allelic effects within families, either by comparing the  
55 separate associations of parentally transmitted and untransmitted alleles with trait values in the offspring  
56 (Spielman et al. 1993; Allison 1997; Eaves et al. 2014; Weiner et al. 2017; Kong et al. 2018), or by  
57 associating differences in siblings' trait values with differences in the alleles they inherited from their  
58 parents (Abecasis et al. 2000; Visscher et al. 2006; Lee et al. 2018). The idea is that, by controlling for  
59 parental genotypes, within-family association studies control for both environmental stratification and  
60 indirect/dynastic effects, while Mendelian segregation randomizes alleles across genetic backgrounds. In  
61 principle, this allows the 'direct genetic effect' of an allele—the causal effect of an allele carried by an  
62 individual on their trait value—to be estimated. Recognizing that a variant detected in a GWAS will  
63 usually not itself be causal for the trait variation but instead will only be correlated with true causal  
64 variants, the direct effect of a genotyped variant is usually interpreted as reflecting the direct causal  
65 effects of nearby loci that are genetically correlated with the focal locus (Young et al. 2019)—but not the  
66 effects of more distant loci that might also be genetically correlated with the focal genotyped locus (e.g.,  
67 because of population structure or assortative mating).

68 Consistent with both the presence of substantial confounds in some population-based GWASs and the  
69 mitigation of these confounds in within-family GWASs, family-based estimates of direct effect sizes and  
70 aggregate quantities based on these estimates (e.g., SNP-based heritabilities) are substantially smaller  
71 than population GWAS estimates for a number of traits, most notably social and behavioural traits (Lee  
72 et al. 2018; Selzam et al. 2019; Mostafavi et al. 2020; Howe et al. 2022; Young et al. 2022). Likewise,  
73 estimates of genetic correlations between traits are sometimes substantially reduced when calculated using  
74 direct effect estimates from within-family GWASs (e.g. Howe et al. 2022). While some of these findings  
75 could reflect the contribution of indirect genetic effects to population GWASs, it is also likely that, at least  
76 for some traits, standard controls for population stratification in population GWASs have been insufficient  
77 (Berg et al. 2019; Sohail et al. 2019; Young et al. 2022; Okbay et al. 2022; Nivard et al. 2022; Border et al.  
78 2022a).

79 Our aim in this paper is to study a general model of confounding in GWASs, to generate clear intuition  
80 for its influence on estimates of effect sizes in both population- and family-based designs. A number of  
81 the issues that we analyze have previously been raised, particularly in the context of population-based  
82 GWASs (e.g., Rosenberg and Nordborg 2006; Platt et al. 2010; Vilhjálmsson and Nordborg 2013; Young  
83 et al. 2019); here, we analyze them in a common framework that allows for comparison of multiple sources  
84 of confounding in both population and family-based GWASs. There is a large literature on GWASs in  
85 non-human organisms (e.g., Atwell et al. 2010; Hayes and Goddard 2010; Peiffer et al. 2014; Josephs et al.  
86 2017). However, although the results and intuition that we derive here apply equally well to human and  
87 non-human GWASs, we shall interpret them primarily from the perspective of human GWASs, in which  
88 the inability to experimentally randomize environments, together with the small effects that investigators  
89 hope to detect, makes confounding a particular concern.

90 Our first focus is on confounding—and genetic confounding in particular—in the absence of  $G \times E$  and  
91  $G \times G$  interactions. To better understand the differences between population and within-family GWASs,

92 we first study a general model of genetic confounding in the absence of  $G \times E$  and  $G \times G$  interactions.  
93 We derive expressions for estimators of direct effects in both population and within-family GWASs, as  
94 functions of the true direct and indirect effects at a locus and the genetic confounds induced by other  
95 loci. In doing so, we find that family-based estimates of direct effects are in fact susceptible to genetic  
96 confounding, contrary to standard interpretation. Reassuringly, in many of the models we consider,  
97 the resulting biases are likely to be small in humans. We also address a related case: family-based  
98 GWAS designs that consider transmitted and untransmitted parental alleles and in which the indirect (or  
99 ‘dynastic’) effect of an allele is estimated from its association with the offspring’s phenotype when carried  
100 by the parent but not transmitted to the offspring. We show that this estimator of indirect effects can be  
101 substantially biased by genetic and environmental confounds, in a similar way to population estimates of  
102 direct effects. Next, we consider various sources of genetic confounding—assortative mating, population  
103 structure, and stabilizing selection on GWAS traits—and how they influence estimates of direct effects  
104 in both population and within-family GWASs.

105 We then turn to sibling indirect effects, which are known to bias estimates of direct effects in sibling-  
106 based GWASs (Young et al. 2019, 2022). We characterize this bias in a simple model, and contrast it to  
107 the bias caused by sibling indirect effects in a population GWAS.

108 Finally, we consider  $G \times E$  and  $G \times G$  interactions, showing how their presence can bias population and  
109 family-based estimates of direct genetic effects in contrasting ways, complicating the interpretation of  
110 family-based estimates.

## 111 2 Effect size estimates in association study designs

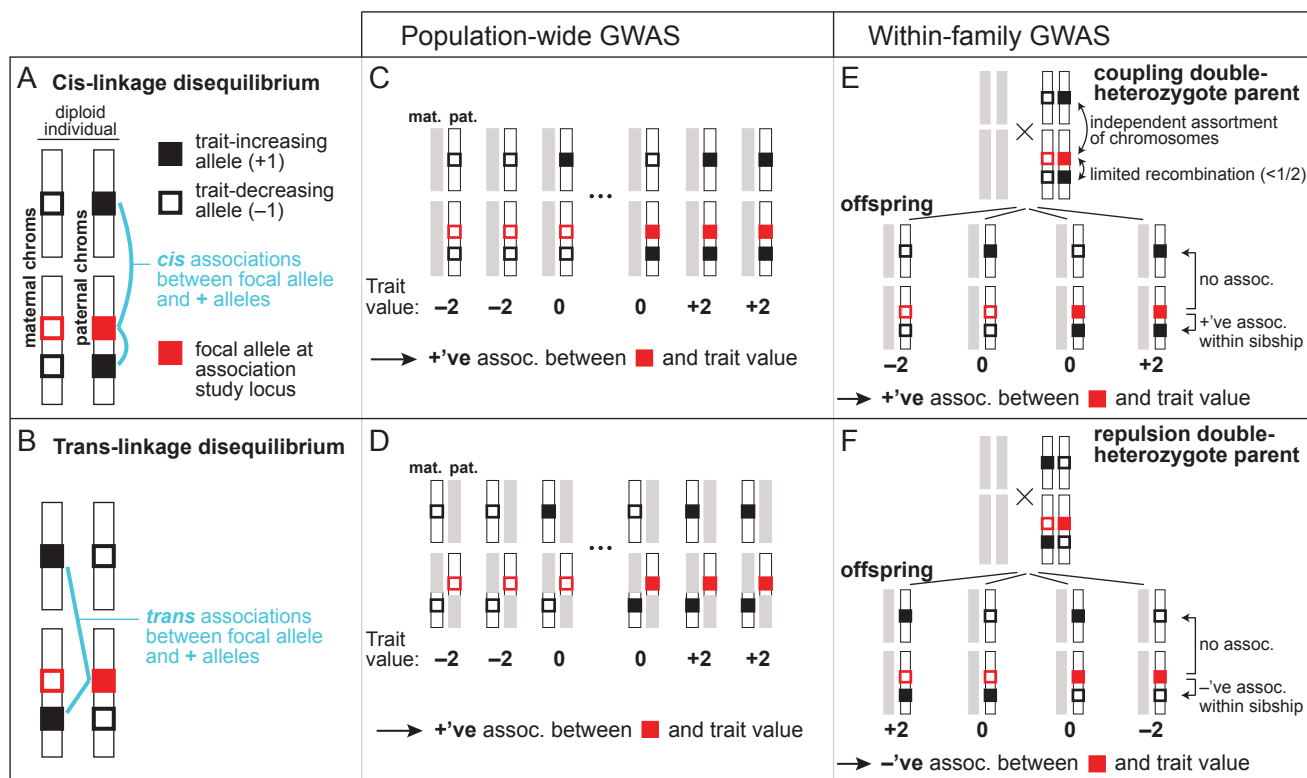
112 Our primary focus will be on how genetic confounding can bias the estimation of direct genetic effects.  
113 These genetic confounds are due to associations between a genotyped variant at a GWAS locus and  
114 causal variants at other loci. As we will see, two kinds of association must be distinguished: cis-linkage  
115 disequilibrium (cis-LD) and trans-linkage disequilibrium (trans-LD). Genetic variants  $A$  and  $B$  are in  
116 positive cis-LD if, when an individual inherits  $A$  from a given parent, the individual is disproportionately  
117 likely to inherit  $B$  from that parent (Fig. 1A).  $A$  and  $B$  are in positive trans-LD if, when an individual  
118 inherits  $A$  from one parent, the individual is disproportionately likely to inherit  $B$  from the other parent  
119 (Fig. 1B). These covariances have also been called gametic and non-gametic LD, respectively (e.g. Weir  
120 2008). To quantify the degrees of cis-LD and trans-LD, we denote by  $D_{ij}$  and  $\tilde{D}_{ij}$  the allelic covariances  
121 between focal variants at loci  $i$  and  $j$  in cis and in trans, and we denote by  $r_{ij}$  and  $\tilde{r}_{ij}$  the analogous  
122 allelic correlation coefficients. For some of our results, it will be important to distinguish the LD present  
123 in the sample on which the association study is performed and the LD present among the parents of the  
124 sample.

125 Consider a trait  $Y$  influenced by genetic variants at a set of polymorphic loci  $L$ , each of which segregates  
126 for two alleles. For ease of interpretation, and without loss of generality, we designate the ‘focal’ allele at  
127 locus  $l \in L$  to be the allele that directly increases the trait value, and we denote by  $p_l$  the frequency of  
128 this allele. Allelic effects are assumed to be additive within and across loci, such that the trait value of  
129 an individual can be written

$$130 \quad Y = Y^* + \underbrace{\sum_{l \in L} g_l \alpha_l^d}_{\text{direct effects}} + \underbrace{\sum_{l \in L} (g_l^m + g_l^f) \alpha_l^i}_{\text{indirect effects}} + \epsilon. \quad (1)$$

131 Here,  $g_l$ ,  $g_l^m$ , and  $g_l^f$  are the numbers (0, 1, or 2) of focal alleles carried at locus  $l$  by the individual, their  
132 mother, and their father respectively,  $\alpha_l^d > 0$  is the direct effect of the focal allele at  $l$ , and  $\alpha_l^i$  is its

133 indirect effect via the maternal and paternal genotypes. (For simplicity, we assume that indirect effects  
 134 via the maternal and paternal genotypes are equal; this assumption is relaxed in Appendix A1.)  $\epsilon$  is the  
 135 environmental noise, with  $\mathbb{E}[\epsilon] = 0$ , and  $Y^*$  is the expected trait value of the offspring of parents who  
 136 carry only trait-decreasing alleles.



**Figure 1:** The influence of cis- and trans-LD on effect size estimates in population-based and within-family association studies. (A) The focal allele at an association study locus (solid red square) is in positive cis-LD with trait-increasing alleles at other loci (solid black squares) if it is disproportionately likely to be found alongside them on an individual’s maternally or paternally inherited genome. (B) The focal allele at the study locus is in positive trans-LD with trait-increasing alleles at other loci if it is disproportionately likely to be found across from them on the maternally and paternally inherited genomes. (C,D) In a population association study, both positive cis- and trans-LD between the focal allele at the study locus and trait-increasing alleles anywhere else in the genome—either on the same chromosome as the study locus or on different chromosomes—generate a spuriously high effect size estimate at the study locus. (E,F) In a sibling association study, a trait-increasing allele causes a spuriously increased effect size estimate at the study locus if the parent is a coupling double heterozygote for the focal and trait-increasing alleles, having inherited them from the same parent (E), but a spuriously decreased estimate if the parent is a repulsion double heterozygote, having inherited them from different parents (F). These biases arise only if the trait-affecting locus is on the same chromosome as the focal study locus. The net bias depends on the relative frequencies of coupling and repulsion double heterozygotes in the parents, which depends on the difference in the degrees of cis- and trans-LD.

## 137 2.1 Population-based association studies

138 The variants at a genotyped locus will usually not themselves have causal effects on the trait, but will  
 139 instead be in cis-LD with—and thus ‘tag’—causal variants at nearby loci. Thus, we typically think of the  
 140 association at a focal genotyped locus as reflecting the direct contributions of a relatively small number of  
 141 tightly linked loci,  $L_{\text{local}}$ , found within tens or perhaps hundreds of kb from the focal locus (Pritchard and  
 142 Przeworski 2001). Under the additive model, therefore, the standard interpretation is that a population  
 143 association study performed at a focal genotyped locus  $\lambda$  provides an estimate of the quantity

$$144 \quad \alpha_\lambda = \frac{1}{p_\lambda(1-p_\lambda)} \sum_{l \in L_{\text{local}}} D_{\lambda l} \alpha_l^d, \quad (2)$$

145 where  $p_\lambda$  is the frequency of the focal allele at  $\lambda$ , and  $D_{\lambda l}$  is the degree of cis-LD between the focal allele  
 146 at  $\lambda$  and a causal allele at a nearby locus  $l \in L_{\text{local}}$ . It is reasonable to think of this quantity as the  
 147 ‘direct effect’ tagged by the focal variant at the genotyped locus  $\lambda$ : in the absence of confounding, it can  
 148 be interpreted as the average phenotypic effect of randomly choosing a non-focal allele in the population  
 149 and swapping it for a focal allele, where in this hypothetical swap, the causal alleles near the locus are  
 150 included. For concreteness, we assume some fixed  $L_{\text{local}}$  in our analyses, but in practice researchers seldom  
 151 have a pre-defined number of ‘local’ SNPs in mind.

152 Effect size estimation in a population GWAS is complicated by the presence of environmental and  
 153 genetic stratification. Under the model in Eq. (1), if we perform a standard population association study  
 154 at locus  $\lambda$ , the estimated effect of the focal allele on the trait Y is

$$155 \quad \hat{\alpha}_\lambda^{\text{pop}} = \frac{2}{V_\lambda} \left( \sum_{l \in L_{\text{local}}} D_{\lambda l} \alpha_l^d + \underbrace{\sum_{l \in L \setminus L_{\text{local}}} D_{\lambda l} \alpha_l^d + \sum_{l \in L} \tilde{D}_{\lambda l} \alpha_l^d}_{\text{genetic confounds, direct}} + \underbrace{\sum_{l \in L} [D'_{\lambda l} + \tilde{D}'_{\lambda l} + 2\tilde{D}_{\lambda l}] \alpha_l^i}_{\text{genetic confounds, indirect}} + \underbrace{\frac{1}{2} \text{Cov}(g_\lambda, \epsilon)}_{\text{environmental confound}} \right), \quad (3)$$

156 where, of the cis- and trans-LD terms,  $D_{\lambda l}$  and  $\tilde{D}_{\lambda l}$  are defined in the GWAS sample while  $D'_{\lambda l}$  and  $\tilde{D}'_{\lambda l}$   
 157 are defined in their parents (Appendix A1.2).  $V_\lambda$  is the genotypic variance at  $\lambda$ , equal to  $2p_\lambda(1-p_\lambda)(1+F_\lambda)$   
 158 where  $F_\lambda$  is Wright’s coefficient of inbreeding at  $\lambda$ .  
 159 where  $F_\lambda$  is Wright’s coefficient of inbreeding at  $\lambda$ .

160 The environmental confound is  $\text{Cov}(g_\lambda, \epsilon)/V_\lambda$ ; all non-local cis- and trans-LD terms in the study  
 161 sample ( $D_{\lambda l}$  and  $\tilde{D}_{\lambda l}$ ,  $l \notin L_{\text{local}}$ ) are direct genetic confounds (Fig. 1C,D); and all cis- and trans-LD  
 162 terms among parents of sampled individuals ( $D'_{\lambda l}$  and  $\tilde{D}'_{\lambda l}$ ), together with all trans-LD terms in the study  
 163 sample ( $\tilde{D}_{\lambda l}$ ), are indirect genetic confounds.

164 The direct genetic confounds arise because an allele carried by an offspring at  $\lambda$  is correlated with  
 165 the alleles that they carry at other loci  $l \in L$  (via  $D_{\lambda l}$  and  $\tilde{D}_{\lambda l}$ ) that directly affect the trait value. The  
 166 indirect genetic confounds arise because an allele carried by the offspring at  $\lambda$ —say, the maternal allele—is  
 167 correlated with alleles carried by the offspring’s mother at other loci ( $D'_{\lambda l}$  and  $\tilde{D}'_{\lambda l}$ ) and alleles carried by  
 168 their father (as reflected by the trans-LD in the offspring,  $\tilde{D}_{\lambda l}$ ). These alleles in the parents can indirectly  
 169 affect the offspring’s trait value.

170 Thus, as is now well appreciated, population-based GWASs potentially suffer from many types of  
 171 confounds (Vilhjalmsson and Nordborg 2013; Young et al. 2019). In practice, they can be reduced by  
 172 including principal components—which capture genome-wide relatedness among GWAS participants—  
 173 as regressors in a GWAS, or by using relatedness matrices in mixed models (Price et al. 2006; Yang  
 174 et al. 2014). However, it is often unclear exactly what these methods control for in a given application

175 (Vilhjálmsson and Nordborg 2013; Young et al. 2019), and they have been shown to be inadequate in  
 176 important cases (e.g., Berg et al. 2019; Sohail et al. 2019). When principal components (or other controls)  
 177 fail to account fully for stratification, then Eq. (3) can be interpreted as a decomposition of the remaining,  
 178 uncontrolled-for confounding in the GWAS.<sup>1</sup>

## 179 2.2 Within-family association studies

180 The two within-family association study designs that we consider are parent-offspring GWASs and sibling  
 181 GWASs. Other designs have been proposed to control for genetic and environmental confounding in the  
 182 estimation of aggregate quantities such as heritability (e.g., Young et al. 2018a), but our primary focus is  
 183 on the estimation of single-marker effect sizes. We do later turn to the interpretation of polygenic score  
 184 regressions within families.

185 **Estimates of direct genetic effects.** Parent-offspring studies can be used to estimate trait associa-  
 186 tions separately for parentally transmitted and untransmitted variants at a locus  $\lambda$ ,  $\hat{\alpha}_\lambda^{(T)}$  and  $\hat{\alpha}_\lambda^{(U)}$ , by  
 187 regressing the trait value  $Y$  on the transmitted and untransmitted genotypes,  $g_\lambda^T$  and  $g_\lambda^U$  (Kong et al.  
 188 2018). The aim is often to estimate the direct effect of a variant,  $\hat{\alpha}_\lambda^d$ , as the difference between these two  
 189 estimates:

$$190 \hat{\alpha}_\lambda^{d,T-U} = \hat{\alpha}_\lambda^{(T)} - \hat{\alpha}_\lambda^{(U)}. \quad (4)$$

191 A second aim is to treat  $\hat{\alpha}_\lambda^{(U)}$  as an estimate of the indirect, or family, effect of the variant. We return to  
 192 this second aim later.

193 In Appendix A1.4, we show that, in the absence of interactions between parental and offspring geno-  
 194 types, the estimate of the direct effect of a variant at locus  $\lambda$  in a parent-offspring study is

$$195 \hat{\alpha}_\lambda^{d,T-U} = \hat{\alpha}_\lambda^{(T)} - \hat{\alpha}_\lambda^{(U)} = \frac{2}{V_\lambda} \sum_{l \in L} (1 - 2c_{\lambda l}) (D'_{\lambda l} - \tilde{D}'_{\lambda l}) \alpha_l^d \quad (5)$$

$$196 \approx \frac{2}{V_\lambda} \left( \sum_{l \in L_{\text{local}}} D'_{\lambda l} \alpha_l^d + \underbrace{\sum_{l \in L \setminus L_{\text{local}}} (1 - 2c_{\lambda l}) (D'_{\lambda l} - \tilde{D}'_{\lambda l}) \alpha_l^d}_{\text{genetic confounds, direct}} \right), \quad (6)$$

197 where  $c_{\lambda l}$  is the sex-averaged recombination rate between  $\lambda$  and  $l$ . The cis- and trans-LD terms  $D'_{\lambda l}$  and  
 198  $\tilde{D}'_{\lambda l}$  are measured in the parents.

200 Similarly, an estimate of the direct effect can be obtained from pairs of siblings by regressing the  
 201 differences in their phenotypes on the differences in their genotypes at the focal locus  $\lambda$ . In the presence  
 202 of genetic confounds, this procedure yields a similar estimate to Eq. (6):

$$203 \hat{\alpha}_\lambda^{d,\text{sib}} \approx \frac{2}{H_\lambda} \left( \sum_{l \in L_{\text{local}}} D'_{\lambda l} \alpha_l^d + \underbrace{\sum_{l \in L \setminus L_{\text{local}}} (1 - 2c_{\lambda l}) (D'_{\lambda l} - \tilde{D}'_{\lambda l}) \alpha_l^d}_{\text{genetic confounds, direct}} \right), \quad (7)$$

<sup>1</sup>By the Frisch-Waugh-Lovell theorem (Greene 2018, pg. 36), Eq. (3) is the estimate one obtains by first regressing both the trait and the focal-locus genotype on the PCs, collecting the residuals from these regressions (which can be thought of as trait and focal-locus genotype values stripped of whatever signal the PCs captured), and regressing the residuals from the trait regression on the residuals from the genotype regression.

204 where  $H_\lambda$  is the fraction of parents who are heterozygous at locus  $\lambda$  (Appendix A1.3). An assumption in  
205 sibling GWASs is that an offspring’s phenotype is not influenced by the genotypes of their siblings—i.e.,  
206 that there are no sibling indirect genetic effects. We consider violations of this assumption later.

207 In Eqs. (6) and (7), there is no environmental confound, because family-based GWASs successfully  
208 randomize the environments of family members with respect to within-family genetic transmission.

209 The derivations above further show that, while population association studies are biased by sums of  
210 trans- and cis-LD between the focal locus and all causal loci (Eq. 3), within-family association studies are  
211 instead biased by *differences* between trans- and cis-LD, and moreover, that the biases in within-family  
212 studies are driven only by LD between the focal locus and causal loci on the same chromosome ( $c_{\lambda l} < 1/2$ ).  
213 To provide an intuition for this result, we focus our discussion on a sibling association study performed  
214 at  $\lambda$ ; the intuition is identical for the analogous parent-offspring study.

215 Because the difference between two siblings in their maternally inherited genotypes is independent  
216 of the difference in their paternally inherited genotypes, we may consider maternal and paternal trans-  
217 missions separately in studying how a locus  $l \in L$  can confound effect size estimation at  $\lambda$  in a sibling  
218 association study. We will phrase our discussion in terms of maternal transmission.

219 For effect size estimation at  $\lambda$  to be genetically confounded by maternal transmission at a distant  
220 locus  $l$ , the mother must be heterozygous at both loci. For if she were homozygous at  $l$ , then maternal  
221 transmission at  $l$  could not contribute to any trait differences between her offspring, while if she were  
222 homozygous at  $\lambda$ , maternal transmission would not result in genetic variation among her offspring at  
223  $\lambda$  with which trait variation could be associated. Therefore, we restrict our focus to mothers who are  
224 heterozygous at both  $\lambda$  and  $l$ , or ‘double heterozygotes’. Two kinds exist (Fig. 1E,F): coupling double  
225 heterozygotes who carry the focal alleles at  $\lambda$  and  $l$  on the same haploid genome (‘in cis’), and repulsion  
226 double heterozygotes who carry them on separate haploid genomes (‘in trans’).

227 We first consider the case where the recombination rate between  $\lambda$  and  $l$  is small ( $c_{\lambda l} \ll 1/2$ ). In  
228 this case, if the mother is a coupling double heterozygote, then her offspring will tend to inherit either  
229 both or neither of the focal alleles at  $\lambda$  and  $l$  (Fig. 1E). Therefore, if one sibling inherits the focal allele  
230 at  $\lambda$  and another does not, the first sibling will tend to inherit the focal (trait-increasing, as we have  
231 defined it) allele at  $l$  and the second sibling will not, so that the effect of locus  $l$  positively confounds the  
232 association between  $\lambda$  and the trait (Fig. 1E). If the mother is instead a repulsion double heterozygote,  
233 then her offspring will tend to inherit either the focal allele at  $\lambda$  or the focal allele at  $l$ , but not both  
234 (Fig. 1F). In this case, if one sibling inherits the focal allele at  $\lambda$  and another does not, the second sibling  
235 will tend to inherit the focal (trait-increasing) allele at  $l$  and the first sibling will not, so that the effect of  
236 locus  $l$  negatively confounds the association between  $\lambda$  and the trait (Fig. 1F). When  $\lambda$  and  $l$  are linked,  
237 therefore, the way in which  $l$  genetically confounds the effect size estimate at  $\lambda$  depends, positively or  
238 negatively, on whether the fraction of coupling double heterozygotes among parents is greater or smaller,  
239 respectively, than the fraction of repulsion double heterozygotes.

240 In contrast, if  $\lambda$  and  $l$  are unlinked ( $c_{\lambda l} = 1/2$ ), then transmissions from coupling and repulsion double  
241 heterozygote parents are equal, and so  $l$  cannot confound estimates at  $\lambda$  (Fig. 1E,F). Put differently,  
242 meiosis in double heterozygotes fully randomizes joint allelic transmissions at  $\lambda$  and  $l$ , with offspring  
243 equally likely to inherit any possible combination of alleles at the two loci.

244 Therefore, only linked loci  $l$  can confound a family-based association study at  $\lambda$ , and they do so in  
245 proportion to (i) how small the recombination rate between  $\lambda$  and  $l$  is, and (ii) the difference between the  
246 fractions of parents who are coupling and repulsion double heterozygotes at  $\lambda$  and  $l$ . Accordingly, if we  
247 write these fractions of parents as  $H_{\lambda l}^{\text{coup}}$  and  $H_{\lambda l}^{\text{rep}}$ , then  $D'_{\lambda l} - \tilde{D}'_{\lambda l} = (H_{\lambda l}^{\text{coup}} - H_{\lambda l}^{\text{rep}})/2$ , and so Eq. (7)



(and Eq. 6) can be rewritten in terms of the relative frequencies of the two kinds of double-heterozygotes:

$$\hat{\alpha}_{\lambda}^{\text{d,sib}} \approx \frac{2}{H_{\lambda}} \left( \sum_{l \in L_{\text{local}}} D'_{\lambda l} \alpha_l^{\text{d}} + \sum_{l \in L \setminus L_{\text{local}}} \left( \frac{1}{2} - c_{\lambda l} \right) (H_{\lambda l}^{\text{coup}} - H_{\lambda l}^{\text{rep}}) \alpha_l^{\text{d}} \right).$$

In a species with many chromosomes, such as humans, for a given locus, there will be many more unlinked loci than linked loci. Therefore, the set of loci that can confound a family-based association study at a given locus will be much smaller than the set of loci that can confound a population association study at the locus. It will often be the case, therefore, that biases in the estimation of direct genetic effects will be smaller in family-based studies than in population studies, a point that we explore below when we consider sources of genetic confounding.

**Estimates of indirect genetic effects.** We now return to the regression of the trait on the untransmitted genotype in parent-offspring GWASs,  $\hat{\alpha}_{\lambda}^{(\text{U})}$ , which has sometimes been treated as an estimate of the indirect effect  $\hat{\alpha}_{\lambda}^{\text{i}}$ . Assuming equal indirect effects via maternal and paternal genotypes (an assumption that we relax in Appendix A1.4),

$$\hat{\alpha}_{\lambda}^{\text{i}} = \hat{\alpha}_{\lambda}^{(\text{U})} = \frac{2}{V_{\lambda}} \left( \underbrace{\sum_{l \in L_{\text{local}}} D'_{\lambda l} \alpha_l^{\text{i}}}_{\text{local indirect effect}} + \underbrace{\sum_{l \in L} (D'_{\lambda l} c_{\lambda l} + \tilde{D}'_{\lambda l} (1 - c_{\lambda l}) + \tilde{D}_{\lambda l}) \alpha_l^{\text{d}}}_{\text{genetic confounds, direct}} + \underbrace{\sum_{l \in L \setminus L_{\text{local}}} D'_{\lambda l} \alpha_l^{\text{i}} + \sum_{l \in L} (\tilde{D}'_{\lambda l} + 2\tilde{D}_{\lambda l}) \alpha_l^{\text{i}}}_{\text{genetic confounds, indirect}} + \underbrace{\frac{1}{2} \text{Cov}(g_{\lambda}^{\text{U}}, \epsilon)}_{\text{environmental confound}} \right). \quad (8)$$

The direct genetic confound reflects associations of the untransmitted alleles at the focal locus with alleles that are transmitted to the offspring at causal loci  $l \in L$  and which directly affect the offspring's trait value (via  $\alpha_l^{\text{d}}$ ). These associations are due to covariances among alleles in each parental genome ( $D'_{\lambda l}$  and  $\tilde{D}'_{\lambda l}$ ) and across the parental genomes (reflected as trans-LD in the offspring,  $\tilde{D}_{\lambda l}$ ). The indirect genetic confound reflects associations of the untransmitted alleles to alleles at other loci in the parents, which can indirectly affect the offspring trait value (via  $\alpha_l^{\text{i}}$ ). Finally, unlike in family-based estimates of direct genetic effects (Eqs. 6 and 7), family-based estimates of indirect effects suffer from environmental confounding, in the same way that population GWASs do (Eq. 3).

Therefore, estimating the indirect effect by regressing the trait value on the untransmitted genotype is highly susceptible to environmental confounding as well as both direct and indirect genetic confounding, in a similar way to estimating the direct effect via a population-based association study (Shen and Feldman 2020). Adjustments for assortative mating in particular have been included in some PGS-based analyses of indirect effects (e.g., Kong et al. 2018; Young et al. 2022). However, it is not clear how robust these adjustments are in the presence of multiple forms of confounding.

### 2.3 Polygenic scores and their phenotypic associations

A current drawback to family-based GWASs is that sample sizes are often small, limiting power to estimate direct genetic effects. Because of this limitation, instead of estimating per-locus effect sizes in family designs, investigators often measure the within-family phenotypic association of a combined linear predictor, a polygenic score (PGS), constructed using effect size estimates across many loci from a population

282 GWAS. In the sibling-based version of this study design, the difference in siblings' population-based PGSs  
283 is regressed on their difference in phenotypes (e.g., Lee et al. 2018; Selzam et al. 2019). In parent-offspring  
284 designs, the population-based PGSs constructed separately for transmitted and untransmitted alleles are  
285 used as linear predictors of the offspring's phenotype, and the difference in their slopes in this regression  
286 is estimated (e.g., Kong et al. 2018; Okbay et al. 2022).

287 When such PGS regressions are used within families for the same phenotype as the population GWAS,  
288 a non-zero slope of the PGS is usually interpreted as reflecting the fact that the PGS—despite having been  
289 calculated from a population GWAS and therefore subject to many potential confounds—nevertheless does  
290 capture the direct genetic effects of alleles. When the PGS for one phenotype is regressed within families  
291 on the value of another phenotype, non-zero slopes are often interpreted as evidence that direct genetic  
292 effects on the two phenotypes are causally related, for example through pleiotropic effects of the alleles  
293 involved.

294 Suppose that we have performed a population GWAS for trait 1, generating effect size estimates  $\hat{\alpha}_\lambda$   
295 at a set of genotyped loci  $\lambda \in \Lambda$ . To construct a PGS for trait 1, these effect size estimates are used as  
296 weights in a linear sum across an individual's genotype:

$$297 \quad PGS_1 = \sum_{\lambda \in \Lambda} g_\lambda \hat{\alpha}_\lambda^{\text{pop}}. \quad (9)$$

298 In a sibling-based study (the results and intuition below will be the same for a parent-offspring study),  
299 the difference between siblings' trait-1 PGSs,  $\Delta PGS_1$ , is regressed on the difference in their values for  
300 trait 2,  $\Delta Y_2$  (note that trait 2 could be the same as trait 1). If  $L$  is the set of loci that causally underlie  
301 variation in trait 2, and  $\beta_l$  are the true effects of variants at these loci on trait 2, then the numerator of  
302 the slope in this regression can be written as

$$303 \quad \text{Cov}(\Delta PGS_1, \Delta Y_2) = 2 \sum_{\lambda \in \Lambda} \sum_{l \in L} (1 - 2c_{\lambda l}) \left( D'_{\lambda l} - \tilde{D}'_{\lambda l} \right) \hat{\alpha}_\lambda^{\text{pop}} \beta_l \quad (10)$$

304 (see Appendix A2). Note that, while the population-based effect size estimates  $\hat{\alpha}_\lambda$  depend on cis- and  
305 trans-LD, as detailed by Eq. (3), the patterns of LD may differ from those in the family study (the  
306  $D'_{\lambda l} - \tilde{D}'_{\lambda l}$  term in Eq. 10) if the population- and family-based studies differ in relevant aspects of sample  
307 composition.

308 The intuition for Eq. (10) is similar to that for the single-locus effect size estimate in a sibling GWAS  
309 (Eq. 7). The numerator of the difference in slopes of transmitted and untransmitted PGSs in a parent-  
310 offspring design takes a similar form to Eq. (10).

311 In the absence of confounding and under some simplifying assumptions, the sibling PGS covariance  
312 measures the contribution of each locus included in the PGS to the additive genic covariance between  
313 traits 1 and 2 that is tagged by the genotyped variants included in the PGS (see Eq. A.23 in Appendix  
314 A2). Under these assumptions, the sibling PGS slope therefore does provide a measure of the underlying  
315 pleiotropy between the traits.

316 Interpretation of the sibling PGS slopes is more complicated in the presence of genetic confounding  
317 (see Eq. A.22 in Appendix A2), which is absorbed into the effect size estimates  $\hat{\alpha}_\lambda^{\text{pop}}$  (Eq. 3) so that the  
318 PGS applies a potentially strange set of weights to the genotyped loci it includes. (A related problem  
319 occurs when indirect genetic effects absorbed by the population-based PGS change the interpretation of  
320 within-family PGS slopes—see Trejo and Domingue (2018); Fletcher et al. (2021).) A non-zero sibling  
321 PGS slope still establishes that the trait-1 PGS loci are in systematic signed intra-chromosomal LD with  
322 loci that causally affect trait 2. However, it no longer necessarily implies that traits 1 and 2 are causally

323 related via pleiotropy, for two reasons. To understand these reasons, suppose that the causal loci for traits  
324 1 and 2 are distinct, i.e., that there is in fact no pleiotropy. First, a SNP included in the trait-1 PGS  
325 could tag local variants that causally affect trait 1 but which are also, via sources of confounding such as  
326 cross-trait assortative mating, in systematic long-range LD with variants on the same chromosome that  
327 causally affect trait 2. Such SNPs will be predictive of sibling differences in trait 2, even though they  
328 locally tag only trait-1 causal variants. Second, LD between variants on the same or distinct chromosomes  
329 that are causal for trait 1 and trait 2 will cause some SNPs that locally tag trait-2 causal variants to be  
330 significantly associated with trait 1 in a population GWAS, and therefore to be included in the trait-1  
331 PGS. These SNPs, since they tag trait-2 causal variants, will be predictive of sibling differences in trait  
332 2.

333 In summary, in the presence of confounding, non-zero sibling PGS slopes cannot be viewed as de facto  
334 evidence for causal relationships between traits.

### 335 **3 Sources of genetic confounding in association studies**

336 As we have seen, genetic confounding of association studies depends, in ways that vary across study  
337 designs, on levels of non-local cis- and trans-LD between the study locus and loci that influence the  
338 study trait. Below, we consider various processes that give rise to non-local cis- and trans-LD, and  
339 their likely impact on the different association study designs. We focus our attention on the potential  
340 for these sources of LD to confound measurement of several key metrics. First, the average deviation  
341 of the estimated effect size from its true value,  $\mathbb{E}[\hat{\alpha}_\lambda - \alpha_\lambda]$ . This measure indicates if effect sizes are  
342 systematically overestimated or underestimated because of genetic confounding. Second, the average  
343 squared effect size estimate, weighted by heterozygosity:  $\mathbb{E}[2p_\lambda(1 - p_\lambda)\hat{\alpha}_\lambda^2]$ . This quantity is related to  
344 important measures such as the genetic variance and SNP-based heritability (Bulik-Sullivan et al. 2015b).  
345 It is also directly related to the variance of effect size estimates, and therefore captures the additional  
346 noise that genetic confounding creates in effect size estimation at a given locus. Finally, if GWASs have  
347 been performed on more than one trait, the covariance across loci of the effect size estimates for two  
348 traits may be of interest. This covariance is determined by the average heterozygosity-weighted product  
349  $\mathbb{E}[2p_\lambda(1 - p_\lambda)\hat{\alpha}_\lambda\hat{\beta}_\lambda]$ , where  $\hat{\alpha}_\lambda$  and  $\hat{\beta}_\lambda$  are the effect size estimates at locus  $\lambda$  for traits 1 and 2.

350 In what follows, for simplicity, we ignore indirect effects and assume that there is no environmental  
351 confounding (i.e., no correlation between genotypes and the environmental effects  $\epsilon$ ). For each of the  
352 sources of genetic confounding that we consider, we calculate the three measures listed above both an-  
353 alytically and in whole-genome simulations carried out in SLiM 4.0 (Haller and Messer 2019). In our  
354 simulations, we use two recombination maps: (i) for illustrative purposes, a simple hypothetical map  
355 where the genome lies along a single chromosome of length 1 Morgan, and (ii) the human linkage map  
356 generated by Kong et al. (2010). A more detailed description of the simulations can be found in the  
357 Methods, and code is available at [github.com/cveller/confoundedGWAS](https://github.com/cveller/confoundedGWAS).

#### 358 **3.1 Assortative mating**

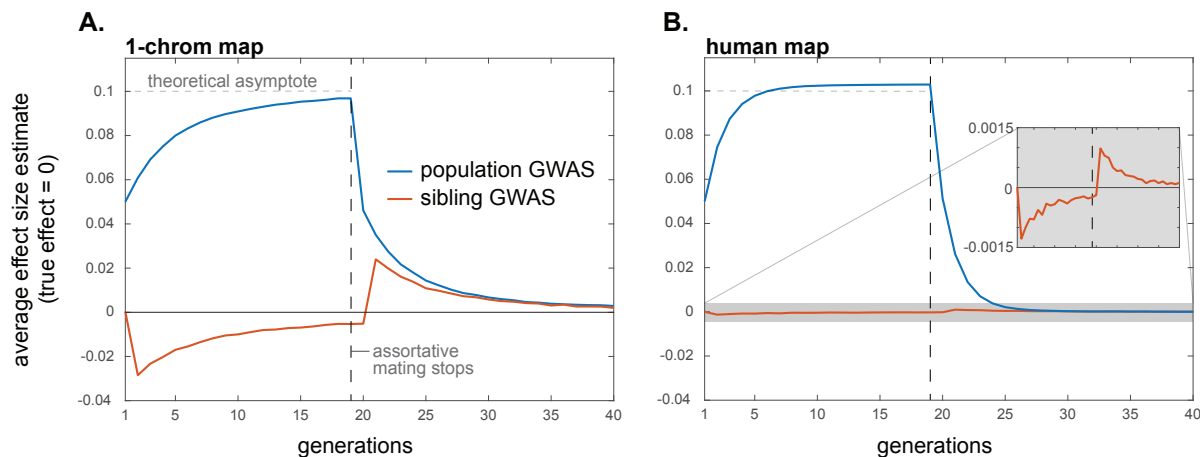
359 Assortative mating is the tendency for mating pairs to be correlated for particular traits—either the  
360 same trait (same-trait assortative mating) or distinct traits (cross-trait assortative mating). For example,  
361 humans are known to exhibit same-trait assortative mating for height and cross-trait assortative mating  
362 for educational attainment and height (amongst many other examples, reviewed in Horwitz and Keller  
363 2022; Border et al. 2022a). Assortative mating generates both cis- and trans-LD: It generates positive

364 trans-LD among trait-increasing alleles because genetic correlations between mates translate to genetic  
365 correlations between maternally and paternally inherited genomes, and it generates positive cis-LD among  
366 trait-increasing alleles because, over generations, recombination converts trans-LD into cis-LD (Crow and  
367 Felsenstein 1968). (In some cases, assortative mating can generate cis-LD by mechanisms additional to  
368 recombination—see Veller et al. 2020.)

369 **Constant-strength assortative mating.** If the strength of assortative mating (measured by the phe-  
370 notypic correlation among mates  $\rho$ ) is constant over time, and there are no other sources of genetic  
371 confounding such as population structure, then, for a given pair of loci  $l, l' \in L$ , the positive cis-LD  $D_{ll'}$   
372 will initially be smaller than the positive trans-LD  $\tilde{D}_{ll'}$ , but will gradually grow towards an equilibrium  
373 value equal to the trans-LD ( $D_{ll'}^* = \tilde{D}_{ll'}^*$ ); in this equilibrium, assortative mating generates new cis-LD at  
374 the same rate as old cis-LD is destroyed by recombination (Crow and Felsenstein 1968, Appendix A3.1).

375 Therefore, in a population GWAS, effect size estimates will initially be biased upwards because of  
376 positive trans-LD, and the magnitude of the bias will grow over time as positive cis-LD too is generated  
377 from this trans-LD (Eq. 3; Fig. 2). In contrast, in a family-based GWAS, effect size estimates will initially  
378 be biased downwards because the positive trans-LD exceeds the positive cis-LD (Eqs. 6 and 7; Fig. 2).  
379 However, as the cis-LD grows over time towards the value of the trans-LD, the magnitude of the downward  
380 bias will shrink, and, in equilibrium, the family-based GWAS will not be confounded by assortative mating  
381 (Fig. 2).

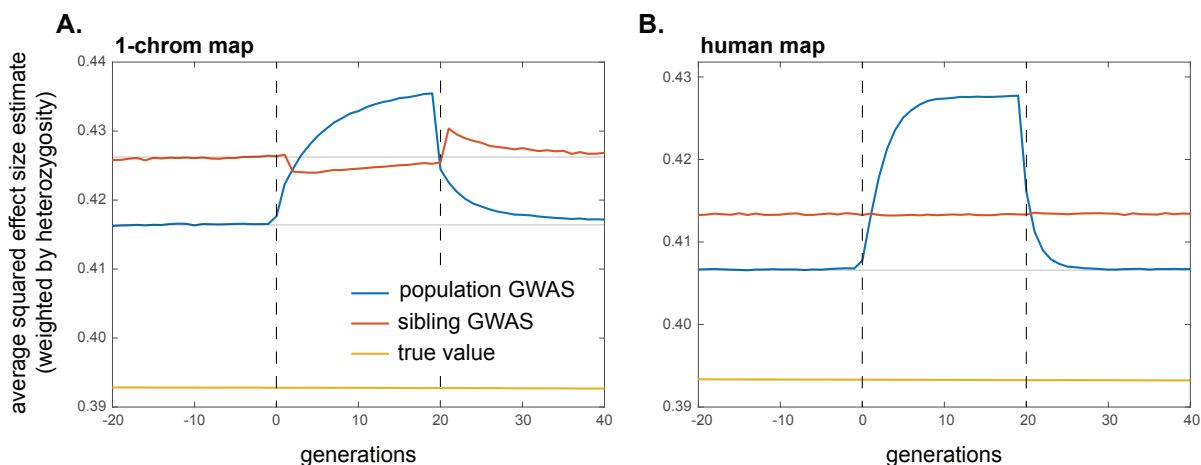
382 Under certain simplifying assumptions, we can calculate the average bias that assortative mating  
383 induces in a population GWAS in equilibrium, in the absence of other sources of genetic confounding such  
384 as population structure (Appendix A3.1). In the case of same-trait assortative mating, effect size estimates



**Figure 2:** Assortative mating systematically biases effect size estimation in population and within-family GWASs, although the bias in within-family GWASs is expected usually to be small. Here, cross-trait assortative mating between traits 1 and 2 occurs for the first 19 generations, after which mating is random. Assortative mating is sex-asymmetric, with strength  $\rho = 0.2$ . Distinct sets of loci underlie variation in trait 1 and 2, with effect sizes at causal loci normalized to 1. Plotted are average estimated effects of the focal alleles at loci causal for trait 1 in population and within-family GWASs on trait 2, for a hypothetical genome with one chromosome of length 1 Morgan (A) and for humans (B). Since the traits have distinct genetic bases, the true effects on trait 2 of the alleles at trait-1 loci are zero. The horizontal lines at 0.1 are a theoretical ‘first-order’ approximation of the asymptotic bias in a population GWAS (Appendix A3.1). Profiles are averages across 10,000 replicate simulation trials. Simulation details can be found in the Methods.

385 are inflated by an average factor of approximately  $h^2\rho/(1 - h^2\rho)$ , where  $\rho$  is the phenotypic correlation  
386 among mates and  $h^2$  is the trait heritability (for similar calculations, see Yengo et al. 2018; Border et al.  
387 2022b). In the case of cross-trait assortative mating, if assortative mating is directional/asymmetric  
388 with respect to sex—i.e., the correlation  $\rho$  is between female trait 1 and male trait 2—then assortative  
389 mating generates spurious associations between trait 1 and alleles that affect trait 2 (and vice versa). If  
390 the loci underlying the two traits are distinct, then, in equilibrium, the spurious effect size estimate at  
391 non-causal loci is approximately  $h^2\rho/2$  times the effect at causal loci, assuming the traits to have the  
392 same heritabilities and genetic architectures (horizontal dashed line in Fig. 2). If cross-trait assortative  
393 mating is bi-directional/symmetric with respect to sex, then, in equilibrium, the average spurious effect  
394 size estimate at non-causal loci is approximately  $h^2\rho$  times the effect at causal loci. Upward biases in  
395 effect size estimates at causal loci are also expected under cross-trait assortative mating, but these are  
396 second-order relative to the biases at non-causal loci (Fig. S1).

397 The systematic over- and under-estimation of effect sizes that assortative mating induces in population  
398 and family-based GWASs, respectively, will also affect our second measure of interest, the heterozygosity-  
399 weighted average squared effect size estimate  $\mathbb{E}[2p_\lambda(1 - p_\lambda)\hat{\alpha}_\lambda^2]$  (and therefore also downstream quantities  
400 such as SNP heritabilities). In a population GWAS, the presence of trans-LD and the gradual creation of  
401 cis-LD under assortative mating will increase the biases in effect size estimates over time (Fig. 2), which  
402 will concomitantly increase the average value of  $\hat{\alpha}_\lambda^2$  (Fig. 3; also see Border et al. 2022b). Moreover, cross-  
403 trait assortative mating will generate signals of genetic correlations among traits even in the absence  
404 of any pleiotropic effects of underlying variants (Border et al. 2022a). In a family-based GWAS, the  
405 temporary attenuation of effect size estimates owing to a transient excess of trans-LD over cis-LD under  
406 assortative mating will lead to a similar attenuation in the average squared effect size estimate (Fig. 3),  
407 although, like the bias in effect size estimates themselves, this attenuation is expected to be small in



**Figure 3:** The impact of assortative mating on the average squared effect size estimate in population and within-family GWASs. Same-trait assortative mating of strength  $\rho = 0.2$  occurs in generations 0–19; mating is random before and after this period. Under random mating, the average squared effect size estimates exceed the true average squared effect size (yellow line) because random drift generates chance local LD with causal alleles that inflates the variance of effect size estimation (e.g. Bulik-Sullivan et al. 2015b). The magnitude of this variance inflation depends on the GWAS design and sample size, and the effect of assortative mating and its cessation should be judged in reference to it. To guide the eye in this judgment, the faint horizontal lines show the average squared effect size estimate in the last 20 generations of the initial burn-in period of random mating. Profiles are averages across 10,000 replicate simulation trials. Simulation details can be found in the Methods.

408 humans (Fig. 3B).

409 As shown by Border et al. (2022a,b), the effects of assortative mating on estimates of heritability and  
410 genetic correlations described above are not well controlled for by LD Score regression (Bulik-Sullivan  
411 et al. 2015a,b). The LD score of a variant proxies the amount of local causal variation the SNP tags, but  
412 because assortative mating generates long-range signed LD among causal variants, it causes local causal  
413 variants to be in long-range signed LD with other causal variants throughout the genome. Therefore,  
414 the slope of the LD score regression absorbs the effects of assortative mating, causing its estimates of  
415 heritability and of the degree of pleiotropy to be inflated.

416 **Historical assortative mating.** If, at some point in time, assortative mating for traits ceases and mat-  
417 ing becomes random with respect to those traits, the positive trans-LD that was present under assortative  
418 mating will immediately disappear, leaving only the positive cis-LD that had built up; this cis-LD will  
419 then be gradually eroded by recombination. If equilibrium had been attained under assortative mating,  
420 the cis-LD would have grown to match the per-generation trans-LD. Therefore, in the first generation  
421 after assortative mating ceases, the upward bias in population GWAS effect size estimates would halve  
422 as the trans-LD disappears (Eq. 3); the bias would then shrink gradually to zero as the cis-LD erodes  
423 (Fig. 2). A similar pattern will be observed for the heterozygosity-weighted average value of  $\hat{\alpha}_\lambda^2$  in the  
424 population GWAS, which eventually returns to its equilibrium level under random mating (Fig. 3).

425 In contrast, with the disappearance of the positive trans-LD but the persistence of positive cis-LD,  
426 the bias in family-based effect size estimates will suddenly become positive once assortative mating ceases  
427 (having temporarily been negative under assortative mating before equilibrium was attained); this bias too  
428 will then gradually shrink to zero as recombination erodes the remaining cis-LD (Fig. 2). Concomitantly,  
429 the average squared effect size estimate in the family GWAS will suddenly increase when assortative  
430 mating ceases, after which it too will gradually return to its equilibrium value under random mating  
431 (Fig. 3).

432 **Assortative mating between traits with different genetic architectures.** An important practical  
433 question is how genetic confounding affects the GWAS loci we prioritize for functional follow-up and for  
434 use in the construction of polygenic scores. SNPs are usually prioritized on the basis of their GWAS  
435 p-value, which is proportional to the estimated variance explained by a SNP,  $2p_\lambda(1 - p_\lambda)\hat{\alpha}_\lambda^2$  (where  $p_\lambda$  is  
436 the minor allele frequency). The results above assume, in the case of cross-trait assortative mating, that  
437 the traits involved have similar genetic architectures (distribution of  $p_l$  and  $\alpha_l$  at causal loci, and the total  
438 number of causal loci). In that case, if there is no pleiotropy between the traits, then while SNPs that  
439 tag trait-1 causal loci are predictive of the value of trait 2 owing to LD between trait-1 and trait-2 causal  
440 loci, we nonetheless expect the SNPs that tag trait-2 causal loci to be better predictors of trait 2, such  
441 that GWAS investigators would primarily pick out SNPs tagging trait-2 causal loci for prioritization and  
442 use in polygenic scores.

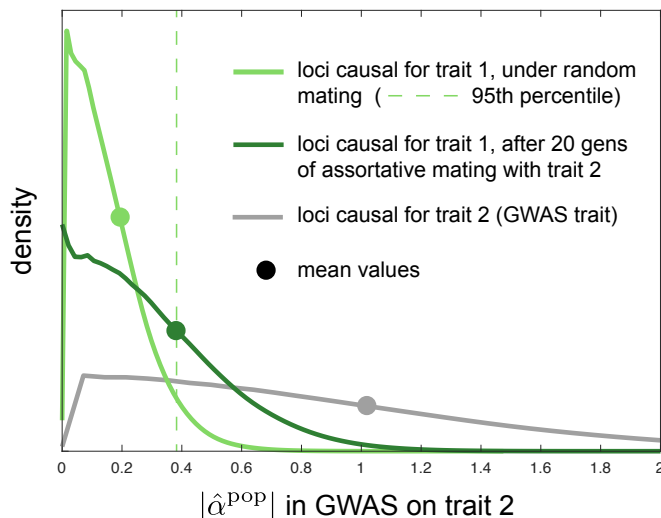
443 However, analysis of human GWASs suggests that quantitative traits can have widely different genetic  
444 architectures, with, in particular, substantial differences in the effective numbers of causal loci involved  
445 and in the distribution of minor allele frequencies (Simons et al. 2022, and references therein). If two  
446 traits with distinct genetic bases show cross-trait assortative mating, but trait 1 has a denser genetic  
447 architecture (fewer causal loci) than trait 2, then the genetic signal of assortative mating—systematic  
448 LD between trait-1 and trait-2 causal loci—will be more heavily loaded per-locus onto trait-1 loci than  
449 onto trait-2 loci. In a GWAS on trait 2, this will inflate the magnitude of spurious effect size estimates  
450 at SNPs that tag trait-1 loci relative to effect size estimates at SNPs that tag causal trait-2 loci. In

451 Appendix A3.1, we quantify this effect, showing that, in a population GWAS for trait 2, the average  
 452 magnitude of spurious effect size estimates at trait-1 loci is proportional to  $\sqrt{|L_2|/|L_1|}$ , where  $|L_1|$  and  
 453  $|L_2|$  are the numbers of loci underlying variation in traits 1 and 2 respectively. Thus, when trait 1 has  
 454 a denser genetic architecture than trait 2 ( $|L_2|/|L_1|$  is large), the magnitudes of effect size estimates at  
 455 non-causal trait-1 loci could substantially overlap with those at causal trait-2 loci (as illustrated in Fig. 4),  
 456 potentially causing part of the apparent, mappable genetic architecture of the trait-2 GWAS to actually  
 457 tag trait-1 loci.

### 458 3.2 Population structure

459 When a population GWAS draws samples from individuals of dissimilar ancestries, differences in the  
 460 distribution of causal genotypes, and potentially of environmental exposures, can confound the association  
 461 study (Lander and Schork 1994; Vilhjálmsson and Nordborg 2013). Correcting for confounds due to  
 462 population structure has therefore been an important pursuit in the GWAS literature (Spielman et al.  
 463 1993; Pritchard et al. 2000; Price et al. 2010).

464 For concreteness, consider a simple model where two populations diverged recently, with no subsequent  
 465 gene flow between them. Genetic drift—and possibly selection—in the two populations will have led to  
 466 allele frequency differences between them at individual loci. If allele frequencies have diverged at both



**Figure 4:** Cross trait assortative mating for traits with different genetic architectures can generate large spurious effect size estimates in population GWASs. Here, the traits have equal heritability, but the number of loci contributing variation to trait 1 is ten-fold smaller than that for trait 2. Shown, for a population GWAS on trait 2, are estimated distributions of the magnitude of effect size estimates at loci causal for trait 2 (grey) and at loci causal for trait 1 (greens), under random mating (light green) and after 20 generations of cross-trait assortative mating (sex-asymmetric, of strength  $\rho = 0.2$ ) for traits 1 and 2 (dark green). Although the true effects of trait-1 loci on trait 2 are zero in these simulations (no pleiotropy), there is sampling noise in effect size estimation at trait-1 loci under random mating (light green line), so that the mean magnitude of effect size estimates is shifted away from zero (light green dot; dashed line displays 95th percentile under random mating). Under assortative mating, the magnitudes of the spurious effect size estimates at trait-1 loci shift significantly rightward (dark green line), coming to overlap substantially with the distribution of effect size estimate magnitudes at causal trait-2 loci (grey line; the distribution for trait-2 loci does not appreciably differ under random and assortative mating). Densities are estimated from pooled effect size estimates from 1,000 replicate simulations. Simulation details in Methods.

467 a genotyped study locus and at loci that causally influence the study trait, these frequency differences  
 468 will manifest as linkage disequilibria between the study locus and the causal loci in a sample taken across  
 469 both populations, even if the loci are not in LD within either population. Specifically, if the frequencies  
 470 of the focal allele at a given locus  $k$  are  $p_k^{(1)}$  and  $p_k^{(2)}$  in populations 1 and 2, then the cis-LD between the  
 471 focal alleles at the association study locus  $\lambda$  and a causal locus  $l$  is

$$472 \quad D_{\lambda l}^{(S)} = \frac{1}{4} \left( p_{\lambda}^{(1)} - p_{\lambda}^{(2)} \right) \left( p_l^{(1)} - p_l^{(2)} \right) \quad (11)$$

473 in a sample that weights the two populations equally, with the superscript  $(S)$  denoting that this LD is  
 474 due to stratification. The trans-LD takes exactly the same form:  $\tilde{D}_{\lambda l}^{(S)} = D_{\lambda l}^{(S)}$ . From Eq. (3), locus  $l$   
 475 therefore confounds estimation of the direct effect at  $\lambda$  in a population GWAS, by an amount proportional  
 476 to

$$477 \quad 2 \left( D_{\lambda l}^{(S)} + \tilde{D}_{\lambda l}^{(S)} \right) \alpha_l^d = \left( p_{\lambda}^{(1)} - p_{\lambda}^{(2)} \right) \left( p_l^{(1)} - p_l^{(2)} \right) \alpha_l^d. \quad (12)$$

478 These genetic confounds are in addition to environmental confounding that would arise if the environments  
 479 of the two populations alter their average trait values by different amounts.

480 In contrast, estimates of direct effects obtained from within-family association studies are not genet-  
 481 ically confounded, because cis- and trans-LD are equal (Eqs. 6 and 7). Another way of seeing this is to  
 482 consider that, by controlling for family, within-family GWASs control for the population, and in the sce-  
 483 nario considered, by construction, there are no within-population LDs to confound effect size estimation.

484 **Allele frequency divergence due to drift.** How do the confounds introduced by population structure  
 485 affect the first of our measures of interest, the average deviation of effect size estimates from their true  
 486 values? The answer depends on the source of allele frequency differences between the two populations. If  
 487 the differences are due to neutral genetic drift, they will be independent of each other (assuming causal  
 488 loci are sufficiently widely spaced) and independent of the direction and size of effects at individual  
 489 loci. Therefore, the LD induced by these allele frequency differences will, on average, not bias effect size  
 490 estimates in a population GWAS:

$$491 \quad \mathbb{E} \left[ \left( p_{\lambda}^{(1)} - p_{\lambda}^{(2)} \right) \left( p_l^{(1)} - p_l^{(2)} \right) \alpha_l^d \right] = \mathbb{E} \left[ p_{\lambda}^{(1)} - p_{\lambda}^{(2)} \right] \mathbb{E} \left[ p_l^{(1)} - p_l^{(2)} \right] \mathbb{E} \left[ \alpha_l^d \right] = 0, \quad (13)$$

492 since  $\mathbb{E} \left[ p_k^{(1)} - p_k^{(2)} \right] = 0$  at any locus  $k$ .

493 However, the LD induced by population structure will inflate the average squared effect size estimate,  
 494 and by extension the variance of effect size estimates (Fig. 5). In Appendix A3.2, we quantify this effect for  
 495 the same simple case of two separate populations. We find that the average squared effect size estimate in  
 496 a population GWAS is an increasing function of the divergence between the two populations (as measured  
 497 by  $F_{ST}$ ), the number of loci contributing variation to the study trait, and the true average squared effect  
 498 size per locus (see also Rosenberg and Nordborg 2006; Lee and Lee 2023a).

499 In contrast, because effect size estimates from within-family GWASs are not confounded in this model  
 500 of isolated populations, the average squared effect size estimate will not differ substantially from its  
 501 expectation in an unstructured population (Fig. 5).

502 While we have focused on a simple model of two isolated populations, the result that within-family  
 503 association studies are not confounded holds for other kinds of population structure as well. Specifically,  
 504 we may be concerned that a population GWAS suffers from genetic confounding along some given axis  
 505 of population stratification. However, the family-based estimates will be unbiased by confounding along  
 506 such an axis if the maternal and paternal genotypes at each locus are exchangeable with respect to each



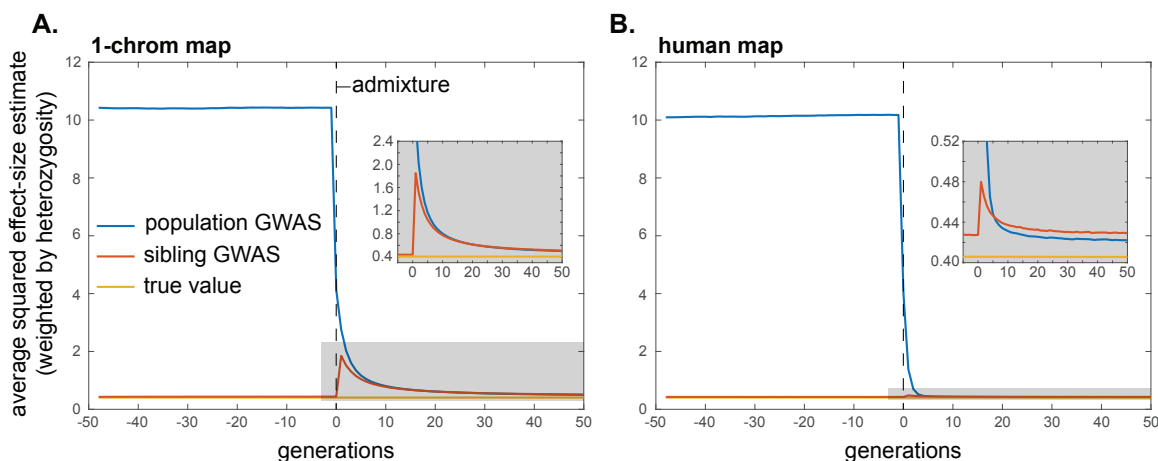
507 other along this axis (Appendix A3.2). This requirement will be met in expectation under many models  
 508 of local genetic drift in discrete populations or along geographic gradients. However, as we will shortly  
 509 argue, migration and admixture introduce further complications.

510 **Allele frequency divergence due to selection or phenotype-biased migration.** Selection and  
 511 phenotype-biased migration can also generate allele frequency differences among populations (for a re-  
 512 view of phenotype-biased migration, see Edelaar and Bolnick 2012). Unlike genetic drift, both of these  
 513 forces can lead to systematic directional associations between effect sizes and changes in allele frequencies  
 514 between populations. For example, if selection has favored alleles that increase the trait in population 1  
 515 but not in population 2, then

$$516 \quad \mathbb{E} \left[ \left( p_l^{(1)} - p_l^{(2)} \right) \alpha_l^d \right] > 0. \quad (14)$$

517 as directional selection causes systematic changes in allele frequencies across the loci  $l$  underlying variation  
 518 in the trait under selection (e.g., Hayward and Sella 2022). Importantly, this form of selection can occur  
 519 even if the mean phenotype of the two populations does not change (Harpak and Przeworski 2021; Yair  
 520 and Coop 2022). Similarly, phenotype-biased migration, where, say, individuals with a higher value of  
 521 the phenotype tend to migrate from population 2 to population 1, can also create a positive association  
 522 between effect sizes and allele frequency differences (Eq. 14).

523 Unlike the case of neutral genetic drift in the two populations, where the sign of the LD between  
 524 two alleles is independent of their effect sizes, the effect-size-correlated associations driven by selection



**Figure 5:** The impact of population structure and admixture on the average squared effect size estimate in population and within-family GWASs. Here, two populations are isolated until generation 0, at which point they mix in equal proportions. Initial allele frequencies are chosen independently for the two populations, such that allele frequency differences between the populations resemble those that would accumulate over time via random drift. As in Fig. 3, the equilibrium value of the mean squared effect size estimate under random mating is greater than the true mean squared effect size, in both the population and within-family GWAS, owing to linkage disequilibria among causal alleles that arise due to drift. This explains why, in the insets, the blue (population) and red (within-family) profiles do not shrink all the way down to the yellow (true) line after admixture, when mating is random. Note too the difference in scale of the y-axes in the insets: the return to equilibrium is much more rapid under the human genetic map than for a hypothetical genome of one chromosome of length 1 Morgan, since, with more recombination, the ancestry-based linkage disequilibria are broken down more rapidly. Profiles are averages across 10,000 replicate simulation trials. Simulation details can be found in the Methods.

525 or phenotype-biased migration can add up across loci, and thus lead to substantial, systematic biases in  
526 estimates of allelic effect sizes. This systematic genetic confounding would also substantially inflate the  
527 average squared effect size estimate and thus measures of the genetic variance tagged by SNPs.

528 In addition, these systematic sources of genetic confounding can generate genetic correlations between  
529 traits with no overlap in their sets of casual loci—i.e., with no pleiotropic relationship. This will occur if  
530 two traits have both experienced selection or biased migration along the same axis. To take a concrete  
531 example, if people tend to migrate to cities in part based on traits 1 and 2, then these traits will become  
532 genetically correlated. If this axis is explicitly included as a covariate in the GWAS, then its influence  
533 on estimates of heritability and genetic correlations will be removed. However, its influence will not be  
534 removed by inclusion of genetic principal components or the relatedness matrix, if this axis (here, city  
535 vs. non-city) is not a major determinant of genome-wide relatedness at non-causal loci (Vilhjálmsón and  
536 Nordborg 2013). Nor will LD score regression control for this influence, as the selection- or migration-  
537 driven differentiation of a variant along the axis will be correlated with the extent to which it tags  
538 long-range causal variants involved in either trait. This effect on LD score regression is similar to that  
539 discussed above for assortative mating (Border et al. 2022a,b). Thus, like assortative mating, selection and  
540 phenotype-biased migration along unaccounted-for axes of population stratification can generate genetic  
541 correlations between traits. These selection- and migration-driven correlations should not necessarily  
542 be viewed as spurious, since genetic correlations should include those that arise from systematic long-  
543 range LD, but they complicate the interpretation of population-level genetic correlations as evidence for  
544 pleiotropy.

545 Again, these issues largely vanish in family-based studies, although phenotype-biased migration can  
546 cause transient differences in cis- and trans-LD that lead to biases in family-based estimates of direct  
547 effects (Eqs. 6 and 7).

### 548 3.3 Admixture

549 When populations that have previously been separated come into contact, alleles from the same ancestral  
550 population remain associated with each other in the admixed population until they are dissociated by  
551 recombination. If allele frequencies had diverged between the ancestral populations, this ‘ancestry disequi-  
552 librium’ can translate to cis-LD between loci affecting a trait (Nei and Li 1973), potentially confounding  
553 GWASs performed in the admixed population. More generally, long range LD will be an issue when there  
554 is genetic stratification and ongoing migration between somewhat genetically distinct groups.

555 For concreteness, we again consider a simple model where two populations have been separated for  
556 some time, allowing allele frequencies to diverge between them. The populations then come into contact  
557 and admix in the proportions  $A$  and  $1 - A$ . We assume that mating is random with respect to ancestry  
558 in the admixed population.

559 Suppose that, just before admixture, the frequencies of the focal allele at a given locus  $k$  were  $p_k^{(1)}$   
560 and  $p_k^{(2)}$  in the two populations. Then the initial degree of cis-LD between loci  $\lambda$  and  $l$  in the admixed  
561 population is given by Eq. (11), weighted by the proportions in which the populations admix:

$$562 \quad D_{\lambda,l,0}^{(A)} = A(1 - A) \left( p_{\lambda}^{(1)} - p_{\lambda}^{(2)} \right) \left( p_l^{(1)} - p_l^{(2)} \right); \quad (15)$$

563 see, e.g., Pfaff et al. (2001). This cis-LD subsequently decays at a rate  $c_{\lambda l}$  per generation, so that,  $t$   
564 generations after admixture,

$$565 \quad D_{\lambda,l,t}^{(A)} = D_{\lambda,l,0}^{(A)} (1 - c_{\lambda l})^t = A(1 - A) \left( p_{\lambda}^{(1)} - p_{\lambda}^{(2)} \right) \left( p_l^{(1)} - p_l^{(2)} \right) (1 - c_{\lambda l})^t. \quad (16)$$

566 Because we assume that mating is random in the admixed population, the trans-LD is zero in every  
567 generation after admixture:  $\tilde{D}_{\lambda,t}^{(A)} = 0$ . Note that the decay of cis-LD in an admixed population will be  
568 slowed if individuals mate assortatively by ancestry, because the trans-LD generated by assortative mating  
569 is continually converted by recombination to new cis-LD (as in our assortative mating model above; see  
570 Zaitlen et al. (2017) for more discussion of this point in the context of population admixture).

571 **Allele frequency divergence due to drift.** How do these patterns of LD affect a population GWAS?  
572 If allele frequency differences between populations arose from neutral drift, they will be independent  
573 of effect sizes at causal loci and across loci, and therefore will not contribute, on average, a systematic  
574 directional bias to effect size estimates. However, they will inflate the average squared effect size estimate,  
575 by a smaller amount than for a population GWAS performed when the populations were still separated  
576 (because of the elimination of trans-LD under random mating in the admixed population). Moreover, this  
577 amount will decline in the generations after admixture as the remaining cis-LD is eroded by recombination  
578 (Eq. 16; Fig. 5). We quantify these effects in Appendix A3.3 (see also Pfaff et al. 2001; Rosenberg and  
579 Nordborg 2006; Zaitlen et al. 2014; Lee and Lee 2023b).

580 Although within-family GWASs were not genetically confounded when the populations were separate  
581 (because cis- and trans-LDs were equal, as discussed above), they become genetically confounded in the  
582 admixed population, as all trans-LD is eliminated by random mating in the admixed population, leaving  
583 an excess of cis-LD relative to trans-LD that biases effect size estimates (Eqs. 6 and 7). As in the case  
584 of the population GWAS, these biases will be zero on average if allele frequency differences between the  
585 ancestral populations were due to drift. However, after admixture, they will still inflate the average  
586 squared effect size estimate (and thus the variance of effect size estimates), which will thereafter decline  
587 in subsequent generations as the cis-LD is gradually broken down by recombination (Eq. 16; Fig. 5).

588 In comparing the average squared effect size estimate in a population and a family-based GWAS, we  
589 observe that the value in the population GWAS rapidly declines to approximately the same level as the  
590 value in the within-family GWAS, despite the former having started at a much higher level in the initial  
591 admixed population (Fig. 5). The explanation is that LD between unlinked loci confounds effect size  
592 estimation in the population GWAS but not the within-family GWAS, such that (i) the average squared  
593 effect size estimate from the population GWAS is initially much higher than that from a within-family  
594 GWAS, because it is inflated by LD between many more pairs of loci, and (ii) the average squared effect  
595 size estimate from the population GWAS declines more rapidly, because LD between unlinked loci is  
596 broken down more rapidly than LD between linked loci.

597 **Allele frequency divergence due to selection or phenotype-biased migration.** In addition to  
598 drift, and as discussed above, selection and phenotype-biased migration can generate systematic, signed  
599 (effect-size correlated) LD, which would lead to systematic cis-LD in the descendent admixed population.  
600 These would lead to larger inflations of genetic variance and genetic correlations than would be expected  
601 had allele frequency divergence between the ancestral populations been due to drift alone, and would  
602 complicate interpretations of genetic correlations as being due to pleiotropy. Moreover, if the admixed  
603 population is more than a few generations old such that LD between unlinked loci but not linked loci  
604 has largely been broken down, then population- and family-based estimates of these quantities might be  
605 similar.

606 **Spurious genetic correlations due to confounding in population-based PGSs.** Factors other  
607 than selection and phenotype-biased migration can also generate non-pleiotropic genetic correlation signals

608 in family-based studies of admixed populations. In fact, the use of confounded population GWAS effect  
609 sizes can be sufficient. As an example of the confounding of genetic correlations in admixed populations  
610 due to a confounded GWAS for one trait, consider the GIANT-GWAS height polygenic score. Owing to  
611 confounding within Europe (Berg et al. 2019; Sohail et al. 2019), the height PGS showed large differences  
612 between Northern Europeans and sets of individuals sampled in other locations, such as the African 1000  
613 genomes samples (Martin et al. 2017). This confounding generated a spurious, systematic correlation  
614 between height effect sizes and allele frequency differences across populations, with height-increasing  
615 alleles that are more common among Northern Europeans being assigned larger effects (Berg et al. 2019).  
616 As a result, in a PGS constructed from these effect size estimates, larger PGS values are predictive of  
617 greater North European ancestry. Now imagine a sibling-based study performed in a sample with recently  
618 admixed ‘European’ and ‘non-European’ ancestry—African Americans, for example. An individual with  
619 a larger value than their sibling for the GIANT height PGS will, on average, carry more ‘European’  
620 ancestry. In African Americans, there will also be a systematic association of lighter skin pigmentation  
621 with recent ‘European’ ancestry, and selection on skin pigmentation will have driven a signed difference  
622 in allele frequencies between European and West African ancestors. Putting these observations together,  
623 the GIANT height PGS, being predictive of the degree of European ancestry, may well be predictive of  
624 skin pigmentation differences between African American sibling pairs (Eq. 10), leading to the naive and  
625 incorrect conclusion that height and skin colour are causally linked. In reality, this result would reflect  
626 the fact that alleles predicted to increase height and alleles that affect skin color are in systematic effect-  
627 signed admixture LD, as in Eq. (15), as a consequence of stratification-biased effect size estimates from  
628 the GIANT European GWAS.

### 629 3.4 Stabilizing selection

630 Stabilizing selection—selection against deviations from an optimal phenotypic value—is thought to be  
631 common (Sella and Barton 2019), and has recently been argued to be consistent with the genetic archi-  
632 tectures of many human traits (Simons et al. 2022). By disfavoring individuals with too many or too  
633 few trait-increasing alleles, stabilizing selection generates negative cis-LD among alleles with the same  
634 directional effect on the trait (Bulmer 1971). Thus, stabilizing selection will attenuate GWAS effect size  
635 estimates at genotyped loci that tag these causal loci.

636 To quantify these biases, we consider the model of Bulmer (1971, 1974), in which a large number  
637 of loci contribute to variation in a trait under stabilizing selection, with the population having adapted  
638 such that the mean trait value is equal to the optimum. Under this model, stabilizing selection rapidly  
639 reduces variance in the trait by generating negative cis-LD among trait-increasing alleles. If we make  
640 the simplifying assumption that all loci have equal effect sizes, then the equilibrium reduction in trait  
641 variance,  $-d^*$  (where  $d^* < 0$ ), can be calculated as a function of the genic variance  $V_g$ , the environmental  
642 noise  $V_E$ , the strength of stabilizing selection  $V_S/V_P$  (scaled according to the phenotypic variance  $V_P$ ),  
643 and the harmonic mean recombination rate,  $\bar{c}_h$ , among loci underlying variation in the trait (Bulmer  
644 1974; Appendix A3.4).

645 Under these same assumptions, we calculate in Appendix A3.4 the average per-locus attenuation  
646 bias in effect size estimates induced by stabilizing selection,  $(\alpha_l - \hat{\alpha}_l)/\alpha_l$ . In a population GWAS, this  
647 attenuation bias is approximately

$$648 \frac{\alpha_l - \hat{\alpha}_l^{\text{pop}}}{\alpha_l} = -\frac{d^*}{V_g}.$$

649 In a within-family GWAS, the average proportionate bias is approximately

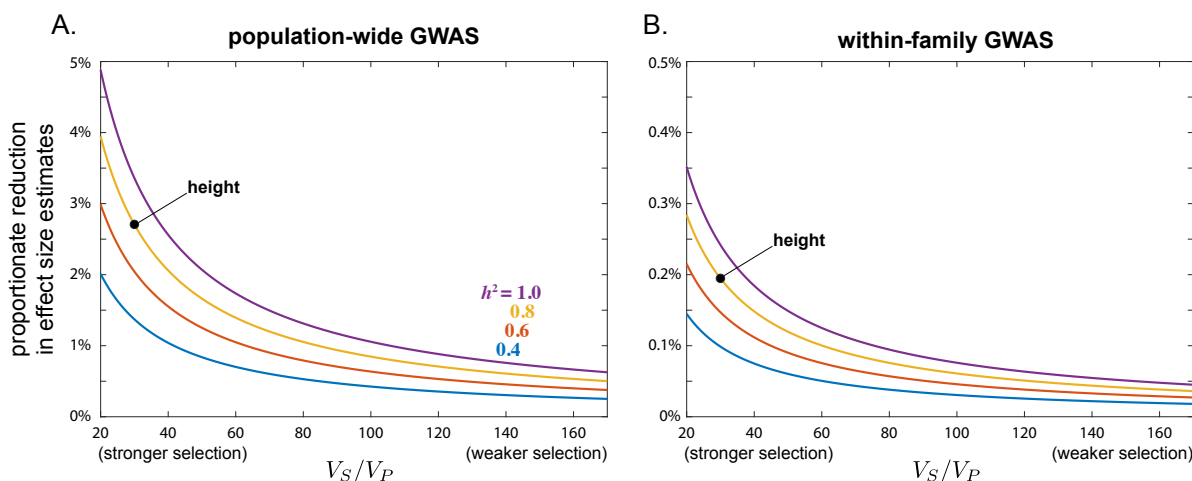
$$650 \frac{\alpha_l - \hat{\alpha}_l^{\text{fam}}}{\alpha_l} = -\frac{d^*(1 - 2\bar{c}_h)}{V_g};$$

651 i.e., smaller than in a population GWAS by a factor of  $1 - 2\bar{c}_h$ .

652 Thus, the bias in effect size estimation can be calculated given estimates of the phenotypic variance and  
 653 heritability of the trait, the harmonic mean recombination rate, and the strength of stabilizing selection  
 654 (Appendix A3.4). In the Methods, making some simplifying assumptions about the genetic architecture  
 655 of the trait in question, we calculate an approximate value  $\bar{c}_h \approx 0.464$  for humans. Using this value,  
 656 Fig. 6 shows the average proportionate reduction in GWAS effect size estimates for various strengths of  
 657 stabilizing selection and heritabilities of the trait. The range of selection strengths was chosen to match  
 658 that inferred for human traits by Sanjak et al. (2018).

659 Attenuation of effect size estimates is larger if stabilizing selection is stronger or if the trait is more  
 660 heritable. Taking height as an example, heritability is  $\sim 0.8$ ,  $V_P \approx 7\text{cm}^2$ , and Sanjak et al. (2018) estimate  
 661 a sex-averaged strength of stabilizing selection of  $V_S/V_P \approx 30$ . From these values, we calculate that a  
 662 population GWAS would systematically underestimate effect sizes at loci that causally influence height  
 663 by about 3% on average, in the absence of other sources of LD (Fig. 6A). More generally, within the  
 664 range of reasonable strengths of stabilizing selection inferred by Sanjak et al. (2018), we calculate average  
 665 attenuations of population-based effect size estimates of up to 5% for highly heritable traits ( $h^2 \approx 1$ )  
 666 under strong stabilizing selection ( $V_S/V_P \approx 20$ ), down to 0.25% for less heritable traits ( $h^2 \approx 0.4$ ) under  
 667 weak stabilizing selection ( $V_S/V_P \approx 170$ ) (Fig. 6A).

668 Given the estimate  $\bar{c}_h \sim 0.464$ , the proportionate bias that stabilizing selection induces in within-  
 669 family GWASs is expected to be a fraction  $1 - 2\bar{c}_h \approx 7\%$  that in population-based GWASs. Thus, for  
 670 height, a within-family GWAS would underestimate effect sizes by only about 0.2% on average (Fig. 6B).



**Figure 6:** Stabilizing selection attenuates GWAS effect size estimates. The calculations displayed here assume that genetic variation in the trait is contributed by 1,000 loci of equal effect spaced evenly along the human genome. Stabilizing selection is stronger if the width of the selection function scaled by the phenotypic variance,  $V_S/V_P$ , is smaller. The placement of the point for human height assumes a heritability of 0.8 and a strength of stabilizing selection of  $V_S/V_P = 30$ , as estimated by Sanjak et al. (2018). Details of these calculations can be found in Appendix A3.4. Note the different scales of the y-axes in A and B.

671 The quantitative importance of these biases will vary by application. In situations where the goal  
672 is gene discovery, for example, 5% reductions in effect size estimates are unlikely to flip the statistical  
673 significance of variants with large effects on a trait. However, the attenuations in effect size estimates  
674 caused by stabilizing selection are systematic across loci, and therefore could substantially affect aggregate  
675 quantities based on these estimates. For example, the range of average reductions in population effect  
676 size estimates calculated above for human traits would translate to reductions in naive estimates of SNP-  
677 based heritabilities of between 0.5% and 10% ( $\sim 6\%$  in the case of height). If effect sizes are estimated by  
678 within-family GWAS, on the other hand, the reductions in these SNP-based heritability estimates would  
679 be much smaller.

680 As a further example, by generating negative LD between alleles with the same directional effect  
681 on the trait, the impact of stabilizing selection opposes, and therefore masks, the genetic impact of  
682 assortative mating (Brown et al. 2016). A practical consequence is that stabilizing selection will tend to  
683 attenuate estimates of the strength of assortative mating based on GWAS effect sizes, which often use  
684 cross-chromosome correlations of polygenic scores (e.g., Yengo et al. 2018; Yamamoto et al. 2023). In  
685 humans, the phenotypic correlation among mates for height has been measured at about  $\sim 0.25$  (Stulp  
686 et al. 2017). In Appendix A3.4, we calculate that estimates of this correlation based on cross-chromosome  
687 correlations in PGSs will be biased downwards by about 20%, to  $\sim 0.20$ , because of stabilizing selection on  
688 height. Were assortative mating weaker, or stabilizing selection stronger, the genetic impact of assortative  
689 mating would be masked to an even greater extent (Appendix A3.4).

690 As in our analysis of assortative mating above, if stabilizing selection ceases in some generation, the  
691 negative LD that built up during the period of stabilizing selection will decay over subsequent generations,  
692 rapidly for pairs of loci on different chromosomes and more slowly for linked pairs of loci. Patterns of  
693 selection on human traits have changed over time—for example, the strength of stabilizing selection on  
694 birth weight has relaxed (Ulizzi and Terrenato 1987). In general, therefore, patterns of confounding reflect  
695 a composite of contemporary and historic processes.

### 696 3.5 Sibling indirect effects

697 Indirect effects of siblings' genotypes on each other's phenotypes are known to be a potential source of  
698 bias in sibling-based GWASs (Fletcher et al. 2021; Young et al. 2022), and can be measured and corrected  
699 only if, in addition to sibling genotypes, parental genotypes are also available (Kong et al. 2018; Young  
700 et al. 2022). To generate intuition for their impact on GWASs, we consider a simple model of indirect  
701 sibling effects in the absence of  $G \times E$  interactions and other confounding effects, focusing on a single-locus  
702 model for simplicity. We suppose that the indirect effect of an individual's phenotype on their sibling's  
703 phenotype is  $\beta$ , so that the phenotypes of two siblings  $i$  and  $j$  can be written

$$\begin{aligned} 704 Y_i &= Y^* + \alpha g_i + \beta Y_j + \epsilon_i, \\ 705 Y_j &= Y^* + \alpha g_j + \beta Y_i + \epsilon_j. \end{aligned} \tag{17}$$

707 Taking their difference and rearranging, we find that

$$708 \Delta Y = \frac{\alpha}{1 + \beta} \Delta g + \frac{1}{1 + \beta} \Delta \epsilon. \tag{18}$$

709 Therefore, in the absence of genetic confounding and  $G \times E$  interactions, a sibling-based association study  
710 would return an effect size estimate of

$$711 \hat{\alpha}^{\text{sib}} = \frac{\alpha}{1 + \beta} \tag{19}$$

712 on average. Thus, if sibling indirect effects are synergistic ( $\beta > 0$ ), they lead to underestimation of the  
 713 direct genetic effect at the locus. In contrast, if sibling indirect effects are antagonistic ( $\beta < 0$ ), they lead  
 714 to overestimation of the direct genetic effect.

715 How would a population GWAS be affected by the same sibling indirect effects? Sibling  $i$ 's phenotype  
 716 can be written

$$\begin{aligned}
 717 \quad Y_i &= Y^* + \alpha g_i + \beta Y_j + \epsilon_i, \\
 718 \quad &= Y^* + \alpha g_i + \beta (Y^* + \alpha g_j + \beta Y_i + \epsilon_j) + \epsilon_i \\
 719 \quad \Rightarrow Y_i &= \frac{1}{1 - \beta^2} (Y^{**} + \alpha g_i + \alpha \beta g_j + \epsilon_i + \beta \epsilon_j), \quad (20) \\
 720
 \end{aligned}$$

721 where  $Y^{**} = (1 + \beta)Y^*$ . Therefore, if we were to randomly choose one sibling from each sibship and  
 722 estimate the effect size at the locus using a population association study across families, we would obtain

$$723 \quad \hat{\alpha}^{\text{POP}} = \frac{\text{Cov}(g_i, Y_i)}{\text{Var}(g_i)} = \frac{1}{1 - \beta^2} (\alpha + \alpha \beta r_g^{\text{sibs}}), \quad (21)$$

725 where  $r_g^{\text{sibs}} = \text{Cov}(g_i, g_j) / \text{Var}(g_i)$  is the genotypic correlation between sibs at the locus. Sibling indirect  
 726 effects alter the effect size estimate in a population GWAS via two channels. The first is through the  
 727 factor  $1/(1 - \beta^2)$  in Eq. (21), which reflects second-order feedbacks of an individual's phenotype on itself,  
 728 via the sibling. Since  $1/(1 - \beta^2) > 1$ , these feedbacks act to exacerbate the effects of causal alleles. For  
 729 example, if sibling indirect effects are antagonistic ( $\beta < 0$ ), then a sibling with a large trait value will tend  
 730 to indirectly reduce the trait value of their sibling, which in turn will indirectly further increase the trait  
 731 value of the focal individual. This channel therefore pushes population GWASs towards overestimating  
 732 the magnitude of direct genetic effects.

733 The other channel by which sibling indirect effects can influence a population GWAS is driven by the  
 734 genotypic correlation among siblings, and is easiest to understand if we assume that sibling indirect effects  
 735 are weak ( $\beta^2 \ll 1$ ). In this case,  $\hat{\alpha}^{\text{POP}} \approx \alpha + \alpha \beta r_g^{\text{sibs}}$ . Since the genotypic correlation  $r_g^{\text{sibs}} > 0$ , this channel  
 736 of sibling indirect effects has the opposite effect to the one it has on a sibling GWAS: if sibling indirect  
 737 effects are synergistic ( $\beta > 0$ ), the population GWAS overestimates the direct genetic effect at the locus,  
 738 while if sibling indirect effects are antagonistic ( $\beta < 0$ ), the population GWAS underestimates the direct  
 739 genetic effect. The reason for this difference is that a sibling GWAS is based on siblings whose genotypes  
 740 differ at the focal locus, and whose genotypic values are therefore anticorrelated. If sibling indirect effects  
 741 are synergistic ( $\beta > 0$ ), they will tend to attenuate the phenotypic differences between such siblings, and  
 742 therefore attenuate effect size estimates. In contrast, because siblings' genotypes are positively correlated  
 743 across the entire population, synergistic sibling indirect effects ( $\beta > 0$ ) will tend to exacerbate phenotypic  
 744 differences across families, leading a population GWAS to overestimate effect sizes.

### 745 3.6 Gene-environment (GxE) and gene-gene (GxG) interactions

746 Up to this point, we have assumed that alleles' direct effects do not vary across environments or genetic  
 747 backgrounds. To generate intuition for the influence of G×E (and G×G) interactions on population  
 748 and family-based GWAS designs, we restrict our focus to a single causal locus, assuming no genetic  
 749 confounding and no indirect effects of siblings. To incorporate G×E interactions, we allow the effect size  
 750 of the alleles at the locus to depend on the family environment. The phenotype of individual  $i$  in family  
 751  $f$  is

$$752 \quad Y_i = Y^* + (\alpha + \alpha_f + \alpha_i) g_i + \epsilon_f + \epsilon_i, \quad (22)$$

753 where we arbitrarily define  $\alpha_f$  and  $\alpha_i$  such that their population means are zero:  $\mathbb{E}[\alpha_f] = \mathbb{E}[\alpha_i] = 0$ .  $\alpha$  is  
754 then the average causal effect of the allele were it randomized across individuals from different families in  
755 our sample.  $\alpha_f$  is the deviation of this effect in family  $f$  due to their environment, and  $\alpha_i$  is an individual  
756 deviation which we assume to be independent of  $i$ 's genotype; both  $\alpha_f$  and  $\alpha_i$  can be thought of as random  
757 slopes in a mixed model. Note that  $\alpha_f$  can reflect the interaction of alleles with the family's external  
758 environment (as we have framed it here) or with the family's genetic background (a  $G \times G$  interaction).

759 If we perform a sibling GWAS by taking pairs of full siblings  $i$  and  $j$  in family  $f$  and regressing  
760 the difference in their phenotypes  $\Delta Y_f = Y_i - Y_j$  on the difference in their genotypes at the focal locus  
761  $\Delta g_f = g_i - g_j$ , we obtain an effect size estimate

$$762 \quad \hat{\alpha}^{\text{sib}} = \alpha + \mathbb{E}[\alpha_f \mid \text{parent heterozygous}], \quad (23)$$

763 where the second term—the deviation of the family-based estimate  $\hat{\alpha}^{\text{sib}}$  from  $\alpha$ —is the average family  
764 deviation conditional on a parent being heterozygous at the focal locus (Appendix A4). The intuition is  
765 that, because only heterozygous parents contribute the genetic variation among siblings on which our effect  
766 size estimate is based, if these heterozygous parents are non-randomly distributed across environments,  
767 then the family-based GWAS samples values from a distribution of family effects  $\alpha_f$  that is different to  
768 the overall population distribution.

769 We can compare this estimate from a sibling GWAS to one from a population GWAS, again under  
770 the assumption of no genetic confounding or indirect effects from siblings:

$$771 \quad \hat{\alpha}^{\text{pop}} \approx \alpha + (1 - 2p)(1 - 2F)\mathbb{E}[\alpha_f \mid g_i = 1] + 2(p + (1 - 2p)F)\mathbb{E}[\alpha_f \mid g_i = 2] \quad (24)$$

772 where  $p$  is the frequency of the focal variant and  $F$  is the inbreeding coefficient at the locus (Appendix  
773 A4). The approximation holds if  $F$  is small. Note that Eq. (24) conditions on the number of focal alleles  
774 carried by the sampled individual, whereas Eq. (23) conditions instead on the parental genotype.

775 Like the family-based estimate, the population-based effect size estimate is distorted when heterozy-  
776 gotes are not randomly distributed over family backgrounds ( $\mathbb{E}[\alpha_f \mid g_i = 1] \neq 0$ ) as well as when homozy-  
777 gotes are not randomly distributed across family backgrounds (when  $\mathbb{E}[\alpha_f \mid g_i = 2] \neq 0$ ). Thus, effect  
778 size estimates from both family- and population-GWAS can differ from the genetic effects that would  
779 be estimated if genotypes were randomly distributed across interacting family backgrounds, and these  
780 distortions will in general not be the same across population and family-based study designs.

781 As noted above, because of current sample size constraints in family-based studies, a common strategy  
782 is to calculate the association of population-based PGSs and phenotypic differences among family mem-  
783 bers. In the absence of confounding, it is clear from Eq. (10) that the influence of  $G \times E$  interactions on the  
784 covariance of sibling differences in PGSs and trait values would depend on the average value of  $\hat{\alpha}^{\text{sib}}\hat{\alpha}^{\text{pop}}$   
785 across loci. Thus the slope of the PGS in this regression could be affected if, on average, the alleles at  
786 casual loci tagged by genotyped variants in the PGS are more often found in environments that suppress  
787 (or enhance) their effects.  $G \times E$  interactions across many loci have been suggested by some recent studies  
788 (Mostafavi et al. 2020; Zhu et al. 2022), but their quantitative impact on differences between population  
789 and family-based GWASs remains unknown.

790 An allele's effect could also systematically differ across families ( $\alpha_f$ ) if it is involved in epistatic  
791 interactions with alleles at other loci in the genome ( $G \times G$ ). By analogy to our  $G \times E$  model above,  
792 epistatic interactions would lead to biases in family-based GWASs if parents who are heterozygous at the  
793 focal study locus tend to have systematically different genotypes at loci that interact epistatically with  
794 the focal locus, relative to the population distribution of such genetic backgrounds.

795 Up to this point, we have also ignored parent-offspring interactions as a possible source of bias in  
796 family-based studies. Following the the same logic above, interactions between parents' and offsprings'



797 alleles will result in family GWAS estimates that are the average effect of the focal allele in an offspring  
798 conditional on the genetic background of a heterozygous parent. Thus, again, a non-random distribution  
799 of genetic backgrounds in heterozygous parents is a potential source of bias.

800 One way that heterozygous parents might exhibit a non-random distribution of genetic backgrounds  
801 is via trait-based assortative mating, which could therefore modify the way that epistasis and parent-  
802 offspring interactions influence effect size estimation in a family-based GWAS relative to a population  
803 GWAS and relative to the true average population effect.

804 A final, overarching complication is that the individuals participating in a population GWAS are not a  
805 random subset of the population(s) from which they are drawn (Fry et al. 2017; Pirastu et al. 2021; Tyrrell  
806 et al. 2021), and families enrolled in GWASs can be even less representative of the population as a whole  
807 (Mostafavi et al. 2020; Benonisdottir and Kong 2022). These participation biases can potentially lead to  
808 systematic differences between the distributions of genotypes and interacting environments experienced  
809 by the population, the GWAS sample, and participants in a family-based study.

## 810 4 Discussion

811 It has long been recognized that population GWASs in humans can be biased by environmental and  
812 genetic confounding (Lander and Schork 1994; Vilhjálmsón and Nordborg 2013). Currently, population  
813 GWASs attempt to control for these confounds by focusing on sets of individuals that are genetically more  
814 similar and by controlling for population stratification. However, these controls are imperfect and are not  
815 always well defined. For example, controlling for genome-wide patterns of population stratification based  
816 on common alleles does not control for the genetic and environmental confounding of rare variants (Zaidi  
817 and Mathieson 2020). Work on genetic confounding has uncovered increasing evidence that assortative  
818 mating may be leading to large biases in estimates of direct genetic effects and to large genetic correlations  
819 for a number of traits (Yengo et al. 2018; Border et al. 2022b,a); moreover, it can often be unclear whether  
820 genetic signals of assortative mating are due to trait-based mate choice or some other more general form  
821 of genetic confounding (e.g., Haworth et al. 2019). Additionally, while we have focused primarily on  
822 genetic confounding, for a number of traits there are also signals of residual environmental confounding  
823 in GWAS signals (Selzam et al. 2019; Mostafavi et al. 2020; Okbay et al. 2022; Abdellaoui et al. 2022).  
824 Thus, subtle and often interwoven forms of genetic and environmental confounding remain a major issue  
825 in many GWASs (Young et al. 2022), compromising the interpretation of GWAS effect size estimates and  
826 downstream quantities such as SNP heritabilities and genetic correlations.

827 Effect size estimates from within-family GWASs are less affected by these various confounds. In  
828 the absence of  $G \times E$  interactions, they are not subject to environmental confounding across families,  
829 because the environments of family members are effectively randomized with respect to within-family  
830 genetic transmission. As we have shown, family-based estimates should also suffer substantially less from  
831 genetic confounding, because genetic transmission at unlinked loci (but not linked loci) is randomized by  
832 independent assortment of chromosomes in meiosis. Nonetheless, family-based GWASs can suffer from  
833 residual genetic confounding as well as sibling indirect effects and  $G \times E / G \times G$  interactions; they also raise  
834 a number of conceptual problems that we discuss below.

835 **Sources of genetic confounding.** Genetic confounding is caused by long-range LD between loci that  
836 affect the trait or traits under study. To illustrate the potential for genetic confounds to bias GWAS  
837 effect size estimates, we have considered several sources of long-range LD. Some of these—assortative  
838 mating, selection on GWAS traits, and phenotype-biased migration—can cause systematic directional

839 biases in GWAS effect size estimates. Others, such as neutral population structure, cause random biases  
840 that influence the variance of effect size estimates and related quantities. Assortative mating and neu-  
841 tral population structure have received considerable theoretical attention in the GWAS literature (e.g.,  
842 Rosenberg and Nordborg 2006; Yengo et al. 2018; Border et al. 2022a,b). Here, we have further outlined  
843 how both selection and phenotyped-biased migration can drive systematic genetic confounding that may  
844 not be well accounted for by current methods of controlling for stratification.

845 We wish to emphasize stabilizing selection in particular as a potential source of systematic confounding  
846 in GWASs. Stabilizing selection has been well studied in the quantitative genetics literature but less so  
847 in the context of GWASs, despite its expected ubiquity. By selecting for compensating combinations of  
848 trait-increasing and trait-decreasing alleles, stabilizing selection generates negative LD between alleles  
849 with the same directional effect on the trait (Bulmer 1971, 1974), and can therefore bias GWAS effect  
850 size estimates downwards. While the potential for stabilizing selection to confound effect size estimation  
851 has been noted (e.g., Brown et al. 2016; Yair and Coop 2022; Li et al. 2023), the resulting biases have  
852 not, to our knowledge, been quantified. Our calculations suggest that these downward biases could,  
853 for some human traits, be as large as 5% systematically across all causal loci in population GWASs.  
854 While biases of this magnitude are unlikely to compromise some goals of GWASs, such as gene discovery,  
855 they could be quantitatively problematic for other GWAS aims, such as estimation of SNP heritabilities  
856 and the strength of assortative mating. Moreover, while our results pertain to (a particular model of)  
857 stabilizing selection, many kinds of selection generate LD between genetically distant loci—in fact, only  
858 multiplicative selection among loci does not (Bürger 2000, pgs. 50 and 177). Therefore, the general result  
859 that selection can generate genetic confounding will hold more broadly.

860 For a given genotyped locus in a GWAS, there is no bright line between local ‘tagged’ LD and long-  
861 range confounding LD, and one reasonable objection to the approach taken here is that that we have used  
862 an arbitrary definition of the causal loci that are locally tagged by a genotyped locus ( $L_{\text{local}}$  in Eq. 2).  
863 All of the sources of genetic confounding that we have considered generate LD among causal loci both  
864 within and across chromosomes. Under these models, the within-chromosome LD that is generated is, in  
865 a sense, a continuation of the LD generated across chromosomes (moving from a recombination rate = 0.5  
866 to  $\leq 0.5$ ). Thus, while investigators may prefer some looser definition of ‘local’ when thinking about  
867 genotyped GWAS loci as tag SNPs, to extend that definition to include all loci on the same chromosome  
868 as the SNP would, by reasonable interpretation, be to include confounding into the desired estimator.

869 The extent to which the absorption of genetic confounding in estimated effect sizes is a problem de-  
870 pends on the application. In the case of polygenic prediction, absorbing environmental effects, indirect  
871 effects, the effects of untyped loci throughout the genome can help to improve prediction accuracy, al-  
872 though this does come at a cost to interpretability. For GWAS applications focused on understanding  
873 genetic causes and mechanisms, the biases in effect size estimates and spurious signals of pleiotropy among  
874 traits generated by genetic confounding will be more problematic.

875 **Indirect genetic effects.** Family GWASs are often interpreted as providing the opportunity to ask to  
876 what extent parental genotypes (or other family genotypes) causally affect a child’s phenotype (‘genetic  
877 nurture’; Kong et al. 2018). Viewed in this way, the association between untransmitted parental alleles  
878 and the child’s phenotype would seem, at first, a natural estimate of indirect genetic effects.

879 In practice, however, if the population GWAS suffers from genetic and environmental confounds, then  
880 the estimated effects of untransmitted alleles will absorb that confounding in much the same way that  
881 estimates of direct genetic effects from a population GWAS do (Eq. 8; Shen and Feldman 2020). For  
882 example, in the case of assortative mating, a given untransmitted allele is correlated with alleles that  
883 were transmitted both by this parent and by their mate, and these transmitted alleles can directly affect

884 the offspring's phenotype. Thus, while family-based estimates of direct genetic effects benefit from the  
885 randomization of meiosis and from controlling for the environment, family-based estimates of indirect  
886 genetic effects lack both of these features and should be interpreted with caution. Indeed, recent work  
887 using parental siblings to control for grandparental genotypes has shown that little of the estimated  
888 'indirect genetic effect' may be causally situated in parents (Nivard et al. 2022). With empirical estimates  
889 of indirect genetic effects potentially absorbing a broad set of confounds (Demange et al. 2022; Young  
890 et al. 2022), and few current studies of indirect effects having designs that allow such confounding to be  
891 disentangled, it is premature—and potentially invalid—to interpret associations of untransmitted alleles  
892 causally in terms of indirect genetic effects (Wolf et al. 1998). Rather, they should be treated agnostically  
893 in terms of 'non-direct' effects.

894 **Direct genetic effects.** Mendelian segregation provides a natural randomization experiment within  
895 families (Fisher 1952), and so crosses in experimental organisms and family designs have long been an  
896 indispensable tool to geneticists in exploring genetic effects and causation. Growing concerns about  
897 GWAS confounding and the increasing availability of genotyped family members have led to a return of  
898 family-based studies to the association study toolkit (Young et al. 2019). Family-based estimates of direct  
899 genetic effects are often interpreted as being unbiased and discussed in terms of the counterfactual effect  
900 of experimentally substituting one allele for another (Morris et al. 2020; Brumpton et al. 2020; Young  
901 et al. 2022).

902 As we have shown, family-based GWASs are indeed less subject to confounding than population-  
903 based GWASs: in the presence of genetic and environmental confounding, the family-based estimate  
904 of the effect size at a given locus provides a much closer approximation to the true effects of tightly  
905 linked causal loci than a population-based estimate does. The family-based estimate is not biased by  
906 environmental variation across families and avoids the correlated effects of the many causal loci that lie  
907 on other chromosomes. Still, the family-based estimate does absorb the effects of non-local causal loci  
908 on the same chromosome, and so cannot truly be said to be free of genetic confounding. Rather than  
909 considering a single allele being substituted between individuals, a better experimental analogy for the  
910 effect size estimate would be to say that we are measuring the mean effect of transmission of a large chunk  
911 of chromosome surrounding the focal locus, potentially carrying many causal loci.

912 In addition, while within-family GWASs offer these advantages, in other ways, they move us further  
913 away from the questions about the sources and causes of variation among unrelated individuals that  
914 motivate population GWASs in the first place. Indeed, the presence of confounding introduces a number  
915 of conceptual issues in moving from within-family GWAS to the interpretation of differences among  
916 individuals from different families (Coop and Przeworski 2022a,b). For example, in the presence of genetic  
917 confounding, the effect of a causal allele of interest will depend on a set of weights: its LD to many other  
918 causal alleles. In estimating the direct effect of the allele, family-based approaches weight these LD terms  
919 differently to population-based approaches, which, we argue, can complicate the interpretation of these  
920 estimates. For example, when previously isolated populations admix, same-ancestry alleles will be held  
921 together in long genomic blocks until these are broken up by recombination, which will happen very  
922 quickly for alleles on different chromosomes but more slowly for alleles on the same chromosome. A  
923 few generations after admixture, therefore, cross-chromosome ancestry LD will largely have dissipated,  
924 but contiguous ancestry tracts will still span substantial portions of chromosome lengths. Since both  
925 population and within-family GWASs are similarly confounded by the same-chromosome LD, their mean  
926 squared effect sizes will be similar in this case (Fig. 5). Bearing in mind that the LD resulting from  
927 admixture is not present in the source populations, it becomes unclear which weighting of ancestry LD  
928 is appropriate if we want to interpret the resulting effect size estimates as direct effects. As this example

929 illustrates, while family-based GWASs are a useful device for dealing with confounding, it is not always  
930 obvious how to interpret the quantities that they measure.

931 A number of additional complications arise when, to compensate for the small effect sizes of individual  
932 loci, researchers combine many SNPs into a polygenic score (PGS) and study the effects of PGSs within  
933 families (or use them as instruments in Mendelian randomization analyses). For one, SNPs are usually  
934 chosen for inclusion in the PGS on the basis of their statistical significance in a population GWAS.  
935 This approach prioritizes SNPs whose effect size estimates are amplified (or even wholly generated) by  
936 confounding (for an example of how this leads to residual environmental confounding in applications of  
937 sibling-based effect size estimates, see Zaidi and Mathieson 2020). Second, the weights given to SNPs that  
938 are included in the PGS absorb the effects of confounding, and this confounding is heterogeneous across  
939 SNPs. Thus, when we study the correlates of trait-A PGS differences between siblings in the presence  
940 of GWAS confounding, we are not observing the average phenotypic outcomes of varying the genetic  
941 component of trait A between siblings. Rather, we are varying a potentially strangely-weighted set of  
942 genetic correlates of trait A.

943 An observation that a population GWAS PGS is predictive of phenotypic differences among siblings  
944 demonstrates that the PGS SNPs tag nearby causal loci, but beyond that, interpretation is difficult.  
945 Notably, if there is cross-trait assortative mating for traits A and B, but no pleiotropic link between the  
946 traits, then some of the SNPs identified as significant in a GWAS on trait A may be tightly linked to  
947 loci that causally affect trait B but not trait A. If these loci are included in the trait-A PGS, then when  
948 we study the effect of variation in the trait-A PGS on sibling differences, we are accidentally absorbing  
949 some components of the variation in trait B across siblings. Thus, we might observe a correlation between  
950 the trait-A PGS and differences in trait B between siblings, and this correlation may be lower than is  
951 observed at the population level, without there existing any pleiotropic (or causal) link between A and B.  
952 These effects can be exacerbated if the two traits have different genetic architectures (Figure 4). Instead  
953 of using a set of SNPs and weights from a population GWAS, genetic correlations between traits due to  
954 pleiotropy could be estimated from the correlation of effect sizes estimated within families (Howe et al.  
955 2022). Given current sample size constraints in family-based studies, the confidence intervals on these  
956 estimates are large. Moreover, significant family-based correlations need not reflect pure pleiotropy, since,  
957 as we have shown, they are not completely free of genetic confounding due to intra-chromosomal LD.

958 Also complicating the interpretation of family-based effect size estimates are various types of interac-  
959 tions. Indirect effects between siblings can bias family estimates of direct genetic effects (Eq. 19; Young  
960 et al. 2019; Fletcher et al. 2021; Young et al. 2022) in ways that are conceptually different from the biases  
961 they introduce to population-based estimates (Eq. 21). These sibling effects can potentially be addressed  
962 with fuller family information (e.g., parental genotypes in addition to sibling genotypes; Kong et al. 2018;  
963 Young et al. 2022).

964 As we have further shown here,  $G \times E$  (and  $G \times G$ ) interactions can also complicate the interpretation  
965 of family-based effect size estimates. The reason is that, even if we were to know the causal alleles  
966 for a trait of interest, what we estimate by measuring their associations with phenotypic differences  
967 within families is not analogous to the counterfactual effects of experimentally substituting alleles in  
968 random individuals. Instead, we are necessarily restricting our focus to the effect of their transmission  
969 from heterozygous parents. If heterozygous parents tend to experience different environments or carry  
970 different genetic backgrounds than homozygotes do, within-family designs will tell us about direct effects  
971 in these particular environments or genetic backgrounds, rather than in the population as a whole. Thus,  
972 although the ongoing shift towards family-based studies is motivated by concerns about confounding, with  
973 different alleles experiencing different environmental and genetic backgrounds, family-based studies can  
974 be influenced by conceptually similar issues of confounding in the presence of  $G \times E$  and  $G \times G$  interactions.

975 Such interactions are difficult to reliably identify and measure, but there are a growing number of potential  
976 examples from GWASs (Tropf et al. 2017; Barcellos et al. 2018; Young et al. 2018b; Mostafavi et al. 2020;  
977 Patel et al. 2022). The interaction issues raised here echo a set of conceptually distinct concerns about  
978 the interpretation of average treatment effects in other contexts (Słoczyński 2022), reinforcing the need  
979 for care in interpreting such estimates as informative about causes across heterogeneous groups.

980 In summary, family-based studies are a clear step forward towards quantifying genetic effects, with  
981 large-scale family studies carrying the potential to resolve long-standing issues in human genetics. How-  
982 ever, these designs come with their own sets of caveats, which will be important to understand and  
983 acknowledge as family-based genetic studies become a key tool in the exploration of causal effects across  
984 disparate fields of study.

985 **Acknowledgements.** We thank Jeremy Berg, Doc Edge, Arbel Harpak, Hanbin Lee, Molly Przeworski,  
986 and members of the Coop lab for helpful discussions and feedback on earlier drafts. Funding was provided  
987 by the National Institutes of Health (NIH R35 GM136290 awarded to GC) and a Branco Weiss fellowship  
988 to CV.

## 989 Methods

990 All simulations were carried out in SLiM 4.0 (Haller and Messer 2019). Code is available at  
991 [github.com/cveller/confoundedGWAS](https://github.com/cveller/confoundedGWAS).

992 For the purpose of carrying out sibling association studies in our simulations, we assumed a simple,  
993 monogamous mating structure: each generation, each female and each male is involved in a single mating  
994 pair, and each mating pair produces exactly two offspring (who are therefore full siblings). To maintain  
995 the precisely even sex ratio required by this scheme, we assumed that a quarter of mating pairs produce  
996 two daughters, a quarter produce two sons, and half produce a son and a daughter. Population sizes  
997 were chosen to ensure that these numbers of mating pairs were whole numbers, and mating pairs were  
998 permuted randomly each generation before assigning brood sex ratios (to ensure that no artifact was  
999 introduced by SLiM's indexing of individuals).

1000 Each generation, per-locus effect size estimates were calculated for both population-wide and sibling  
1001 GWASs. The former were calculated as the regression of trait values on per-locus genotypes, while  
1002 the latter were calculated as the regression of sibling differences in trait values on sibling differences in  
1003 per-locus genotypes.

1004 In all simulations, the total population size was  $N = 40,000$ .

1005 **Assortative mating.** For our general cross-trait assortative mating setup, traits 1 and 2 are influenced  
1006 by variation at sets of bi-allelic loci  $L_1$  and  $L_2$  respectively. The effect sizes of the reference allele at locus  $l$   
1007 on traits 1 and 2 are  $\alpha_l$  and  $\beta_l$  respectively. An individual's polygenic score (PGS) is then  $P_1 = \sum_{l \in L_1} g_l \alpha_l$   
1008 for trait 1 and  $P_2 = \sum_{l \in L_2} g_l \beta_l$  for trait 2. In all the scenarios we simulated, traits had heritability 1, so  
1009 that individuals' trait values are the same as their PGSs.

1010 Our aim is to simulate a scenario where assortative mating is based on females' values for trait 1 and  
1011 males' values for trait 2, such that, across mating pairs, the correlation of the mother's PGS for trait 1,  
1012  $P_1^m$ , and the father's PGS for trait 2,  $P_2^f$ , is a constant value  $\rho$  (in all of our simulations,  $\rho = 0.2$ ). To  
1013 achieve this, we use an algorithm suggested by Zaitlen et al. (2017): At the outset, we choose an accuracy  
1014 tolerance  $\varepsilon$  such that, if by some assignment of mates the correlation of their PGSs falls within  $\varepsilon$  of the  
1015 target value  $\rho$ , we accept that assignment. Each generation in which assortative mating occurs, we rank  
1016 females in order of their PGSs for trait 1, and males in order of their PGSs for trait 2. We then calculate  
1017 the PGS correlation across mating pairs,  $\rho_0$ , if females and males were matched according to this ranking.  
1018 If this (maximal) correlation is smaller than the upper bound of our target window ( $\rho_0 < \rho + \varepsilon$ , which  
1019 very seldom occurred in our simulations), then females and males mate precisely according to their PGS  
1020 rankings and we move on to the next generation. If, instead,  $\rho_0$  exceeds  $\rho + \varepsilon$ , then we follow the following  
1021 iterative procedure until we have found a mating structure under which the correlation of PGSs falls  
1022 within  $\varepsilon$  of the target value  $\rho$ .

1023 First, we choose initial 'perturbation sizes'  $\xi_0$  and  $\xi_1 = 2\xi_0$ . Suppose that, in iteration  $k$  of the  
1024 procedure, the perturbation size is  $\xi_k$  and the chosen mating structure leads to a correlation among mates  
1025 of  $\rho_k$ . If  $|\rho_k - \rho| < \varepsilon$ , we accept the mating structure and move on to the next generation. Otherwise,  
1026 we choose a new perturbation size  $\xi_{k+1}$ : (i) if  $\rho_{k-1}, \rho_k > \rho$ , then  $\xi_{k+1} = 2\xi_k$ ; (ii) if  $\rho_{k-1} > \rho > \rho_k$  or  
1027  $\rho_{k-1} < \rho < \rho_k$ , then  $\xi_{k+1} = (\xi_{k-1} + \xi_k)/2$ ; (iii) if  $\rho_{k-1}, \rho_k < \rho$ , then  $\xi_{k+1} = \xi_k/2$ . Once we have chosen  
1028  $\xi_{k+1}$ , for each individual we perturb their PGS (trait 1 for females; trait 2 for males) by a value chosen  
1029 from a normal distribution with mean 0 and standard deviation  $\xi_{k+1}$ , independently across individuals.  
1030 We then rank females and males according to their perturbed PGSs, and calculate the correlation  $\rho_{k+1}$   
1031 of their true PGSs if they mate according to this ranking. (Since, in our experience, there can be substantial  
1032 variance in the  $\rho_{k+1}$  values that result from this procedure, we repeat it 5 times and choose the mating

1033 structure that produces the value of  $\rho_{k+1}$  closest to the target value  $\rho$ .) We then decide if another  
1034 iteration—i.e., another perturbation size  $\xi_{k+2}$ —is required.

1035 **Fig. 2. Cross-trait assortative mating for traits with the same genetic architecture.** In the  
1036 simulations displayed in Fig. 2,  $\rho = 0.2$ , and traits 1 and 2 have identical but non-overlapping genetic  
1037 architectures:  $L_1$  and  $L_2$  are distinct sets of 500 loci each, with  $\alpha_l = 1$  and  $\beta_l = 0$  for  $l \in L_1$ , and  
1038  $\alpha_l = 0$  and  $\beta_l = 1$  for  $l \in L_2$ . Loci in  $L_1$  and  $L_2$  alternate in an even spacing along the physical (bp)  
1039 genome. Fig. 2A shows results for the ‘single chromosome’ case where the recombination fraction between  
1040 adjacent loci is  $c = 1/999$  in both sexes (such that the single-chromosome genome receives, on average,  
1041 one crossover per transmission). Fig. 2B shows results for the case where recombination fractions between  
1042 loci are calculated from the human female and male linkage maps generated by Kong et al. (2010). In  
1043 both cases, we assumed no crossover interference.

1044 At each locus, the initial frequency of the reference allele was  $1/2$ , with reference alleles assigned  
1045 randomly across diploid individuals and independently across loci such that, in expectation, Hardy-  
1046 Weinberg and linkage equilibrium initially prevail. The assortative mating algorithm above was run  
1047 for 19 generations, with a target correlation  $\rho = 0.2$ , a tolerance parameter  $\varepsilon = \rho/100$ , and an initial  
1048 perturbation size  $\xi_0 = 4 \left[ \max \left( \{ \{P_1^m\}, \{P_2^f\} \} \right) - \min \left( \{ \{P_1^m\}, \{P_2^f\} \} \right) \right]$ . Thereafter, assortative mating  
1049 was switched off, with mating pairs (still monogamous) being chosen randomly.

1050 **Fig. 3. Same-trait assortative mating.** The algorithm we followed to ensure assortative mating  
1051 of a given strength was the same as that for Fig. 2 above, but here traits 1 and 2 are identical. 1,000  
1052 loci underlie variation in the trait, and are evenly spread along the physical genome. The effect size  
1053 of the reference allele at each locus was drawn from a normal distribution with mean 0 and standard  
1054 deviation 1, independently across loci. The initial frequency of the reference allele at each locus was  
1055 drawn, independently across loci, from a uniform distribution on  $[MAF, 1 - MAF]$ ; in our simulations,  
1056 we chose a minimum minor allele frequency of  $MAF = 0.1$ . Since here we are interested in quantifying  
1057 the mean squared effect size estimate, which is directionally affected by drift-based local LD that may not  
1058 be present in our initial configuration, we allowed 150 generations of random mating before switching on  
1059 assortative mating (only the final 20 generations of this random mating burn-in are displayed in Fig. 3).  
1060 Assortative mating occurred for 19 generations, after which random mating occurred for a further 20  
1061 generations.

1062 **Fig. 4. Cross-trait assortative mating for traits with different architectures.** We again followed  
1063 a similar procedure to that for Fig. 2 above, but now, while traits 1 and 2 have distinct genetic bases, the  
1064 numbers of loci contributing variation to traits 1 and 2 are  $|L_1| = 100$  and  $|L_2| = 1,000$ . Trait-1 loci are  
1065 placed evenly along the physical genome, with trait-2 loci then evenly spaced among the trait-1 loci; we  
1066 used the human linkage map for these simulations. At both trait-1 and trait-2 loci, the initial frequency  
1067 of the focal allele was drawn from a uniform distribution on  $[MAF, 1 - MAF]$ , with  $MAF = 0.1$ . At trait-  
1068 2 loci, true effect size were randomly drawn from a normal distribution with mean zero and standard  
1069 deviation 1; at trait-1 loci, true effect sizes were randomly drawn from a normal distribution with mean  
1070 zero and standard deviation  $\sqrt{10}$ , so that traits 1 and 2 have equal genic variances. After a burn-in of 150  
1071 generation of random mating, assortative mating was switched on. We performed a population GWAS at  
1072 the end of the period of random mating and after 20 generations of assortative mating. These GWASs  
1073 were performed across 1,000 replicate trials, with the effect size estimates then pooled across trials. From

1074 these, we estimated the densities of the absolute values of effect size estimates using Matlab’s kernel  
1075 density estimator `ksdensity`, specifying that the support of the distributions be positive.

1076 **Fig. 5. Population structure and admixture.** We wished first to simulate a situation where two  
1077 populations of size  $N/2$  have been separated for a length of time such that the value of  $F_{ST}$  between them  
1078 is some predefined level (in our case, a mean  $F_{ST}$  per locus of 0.1). To do so without having to run the  
1079 full population dynamics of two allopatric populations for a prohibitively large number of generations, we  
1080 simply assigned allele frequencies to achieve the desired level of  $F_{ST}$ . We assumed 1,000 loci spread evenly  
1081 over the physical genome. At each locus  $l$ , we chose an ‘ancestral’ frequency  $p_l^a$  for the reference allele  
1082 independently from a uniform distribution on  $[MAF, 1 - MAF]$ , with  $MAF = 0.2$ . We then perturbed  
1083 this allele frequency in populations 1 and 2 by independent draws from a normal distribution with mean  
1084 0 and variance  $2p_l^a(1 - p_l^a)F_{ST}$ ; if a perturbed allele frequency fell below 0 or above 1, we set it to 0 or 1  
1085 respectively. The population dynamics described above, with monogamous mating pairs chosen randomly,  
1086 were then run for 50 generations.

1087 In generation 50, the two populations merge, forming an admixed population of size  $N$ . The same  
1088 population dynamics, with monogamous mating pairs chosen randomly, were then run for a further 50  
1089 generations.

1090 **Fig. 6. Stabilizing selection** To calculate the bias in GWAS effect size estimation caused by stabilizing  
1091 selection, we must first calculate the harmonic mean recombination rate. We focus on humans, and  
1092 consider only the autosomal genome. The set of loci underlying variation in the trait is  $L$ , which we  
1093 apportion among the 22 autosomes according to their physical (bp) lengths (as reported in GRCh38.p11 of  
1094 the human reference genome; <https://www.ncbi.nlm.nih.gov/grc/human/data?asm=GRCh38.p11>). For  
1095 each chromosome, we spread its allotment of loci evenly over its sex-averaged genetic (cM) length, using  
1096 the male and female linkage maps produced by Kong et al. (2010). (We use genetic lengths instead of  
1097 physical lengths because, were we to spread loci evenly over the physical lengths of the chromosomes,  
1098 some pairs of adjacent loci on some chromosomes might have a sex-averaged recombination fraction of 0,  
1099 in which case the harmonic mean recombination rate would be undefined.) For each pair of linked loci,  
1100 the recombination rate between them was estimated separately from the male and female genetic distance  
1101 between them using Kosambi’s map function (Crow 1990). Pairs of loci on separate chromosomes have a  
1102 recombination fraction of  $1/2$ . With the sex-averaged recombination fraction  $c_{ll'}$  thus calculated for every  
1103 pair of loci  $(l, l')$ , the harmonic mean recombination fraction was calculated as  $\bar{c}_h = \binom{|L|}{2} / \left( \sum_{l, l'} \frac{1}{c_{ll'}} \right)$ ,  
1104 where  $\binom{|L|}{2} = |L|(|L| - 1)/2$  is the number of pairs of distinct loci in  $L$ .

1105 Performing this calculation with  $|L| = 1,000$  loci, we obtain an estimate of  $\bar{c}_h \approx 0.464$  for human  
1106 autosomes. Substituting this estimate into Appendix Eqs. (A.87) and (A.88) then defines the curves  
1107 plotted in Figs. 6A and 6B respectively.



## 1108 References

- 1109 Abdellaoui, A., Dolan, C. V., Verweij, K. J., and Nivard, M. G. (2022). Gene–environment correlations  
1110 across geographic regions affect genome-wide association studies. *Nature Genetics*, 54(9):1345–1354.
- 1111 Abecasis, G. R., Cardon, L. R., and Cookson, W. O. C. (2000). A general test of association for quanti-  
1112 tative traits in nuclear families. *American Journal of Human Genetics*, 66(1):279–292.
- 1113 Allison, D. B. (1997). Transmission-disequilibrium tests for quantitative traits. *American Journal of*  
1114 *Human Genetics*, 60(3):676–690.
- 1115 Atwell, S., Huang, Y. S., Vilhjálmsdóttir, B. J., Willems, G., Horton, M., Li, Y., Meng, D., Platt, A., Tarone,  
1116 A. M., Hu, T. T., et al. (2010). Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana*  
1117 inbred lines. *Nature*, 465(7298):627–631.
- 1118 Barcellos, S. H., Carvalho, L. S., and Turley, P. (2018). Education can reduce health differences related  
1119 to genetic risk of obesity. *Proceedings of the National Academy of Sciences*, 115(42):E9765–E9772.
- 1120 Benonisdóttir, S. and Kong, A. (2022). The genetics of participation: method and analysis. *bioRxiv*, doi:  
1121 <https://doi.org/10.1101/2022.02.11.480067>.
- 1122 Berg, J. J., Harpak, A., Sinnott-Armstrong, N., Joergensen, A. M., Mostafavi, H., Field, Y., Boyle, E. A.,  
1123 Zhang, X., Racimo, F., Pritchard, J. K., et al. (2019). Reduced signal for polygenic adaptation of  
1124 height in UK Biobank. *eLife*, 8:e39725.
- 1125 Border, R., Athanasiadis, G., Buil, A., Schork, A., Cai, N., Young, A., Werge, T., Flint, J., Kendler, K.,  
1126 Sankararaman, S., W, D. A., and A, Z. N. (2022a). Cross-trait assortative mating is widespread and  
1127 inflates genetic correlation estimates. *Science*, 378(6621):754–761.
- 1128 Border, R., O’Rourke, S., de Candia, T., Goddard, M. E., Visscher, P. M., Yengo, L., Jones, M., and  
1129 Keller, M. C. (2022b). Assortative mating biases marker-based heritability estimators. *Nature Com-*  
1130 *munications*, 13(1):1–10.
- 1131 Brown, B. C., Price, A. L., Patsopoulos, N. A., and Zaitlen, N. (2016). Local joint testing improves power  
1132 and identifies hidden heritability in association studies. *Genetics*, 203(3):1105–1116.
- 1133 Brumpton, B., Sanderson, E., Heilbron, K., Hartwig, F. P., Harrison, S., Vie, G. Å., Cho, Y., Howe, L. D.,  
1134 Hughes, A., Boomsma, D. I., et al. (2020). Avoiding dynastic, assortative mating, and population strat-  
1135 ification biases in Mendelian randomization through within-family analyses. *Nature Communications*,  
1136 11(1):3519.
- 1137 Bulik-Sullivan, B. (2015). Relationship between LD score and Haseman-Elston regression. *BioRxiv*, doi:  
1138 <https://doi.org/10.1101/018283>.
- 1139 Bulik-Sullivan, B. K., Finucane, H. K., Anttila, V., Gusev, A., Day, F. R., Loh, P.-R., Duncan, L., Perry,  
1140 J. R., Patterson, N., Robinson, E. B., et al. (2015a). An atlas of genetic correlations across human  
1141 diseases and traits. *Nature Genetics*, 47(11):1236–1241.
- 1142 Bulik-Sullivan, B. K., Loh, P.-R., Finucane, H. K., Ripke, S., Yang, J., Patterson, N., Daly, M. J., Price,  
1143 A. L., and Neale, B. M. (2015b). LD Score regression distinguishes confounding from polygenicity in  
1144 genome-wide association studies. *Nature Genetics*, 47(3):291–295.

- 1145 Bulmer, M. G. (1971). The effect of selection on genetic variability. *American Naturalist*, 105(943):201–  
1146 211.
- 1147 Bulmer, M. G. (1974). Linkage disequilibrium and genetic variability. *Genetics Research*, 23(3):281–289.
- 1148 Bürger, R. (2000). *The Mathematical Theory of Selection, Recombination, and Mutation*. Wiley, Chich-  
1149 ester, UK.
- 1150 Coop, G. and Przeworski, M. (2022a). Lottery, luck, or legacy. A review of “The Genetic Lottery: Why  
1151 DNA matters for social equality”. *Evolution*, 76(4):846–853.
- 1152 Coop, G. and Przeworski, M. (2022b). Luck, lottery, or legacy? The problem of confounding. A reply to  
1153 Harden. *Evolution*, 76(10):2464–2468.
- 1154 Crow, J. F. (1990). Mapping functions. *Genetics*, 125(4):669–671.
- 1155 Crow, J. F. and Felsenstein, J. (1968). The effect of assortative mating on the genetic composition of a  
1156 population. *Eugenics Quarterly*, 15(2):85–97.
- 1157 Crow, J. F. and Kimura, M. (1970). *An Introduction to Population Genetics Theory*. Harper and Row,  
1158 New York.
- 1159 Demange, P. A., Hottenga, J. J., Abdellaoui, A., Eilertsen, E. M., Malanchini, M., Domingue, B. W.,  
1160 Armstrong-Carter, E., De Zeeuw, E. L., Rimfeld, K., Boomsma, D. I., et al. (2022). Estimating effects  
1161 of parents’ cognitive and non-cognitive skills on offspring education using polygenic scores. *Nature*  
1162 *Communications*, 13(1):4801.
- 1163 Eaves, L. J., Pourcain, B. S., Smith, G. D., York, T. P., and Evans, D. M. (2014). Resolving the  
1164 effects of maternal and offspring genotype on dyadic outcomes in genome wide complex trait analysis  
1165 (“M-GCTA”). *Behavior Genetics*, 44:445–455.
- 1166 Edelaar, P. and Bolnick, D. I. (2012). Non-random gene flow: an underappreciated force in evolution and  
1167 ecology. *Trends in Ecology & Evolution*, 27(12):659–665.
- 1168 Ewens, W. J. and Spielman, R. S. (1995). The transmission/disequilibrium test: history, subdivision, and  
1169 admixture. *American Journal of Human Genetics*, 57(2):455–464.
- 1170 Felsenstein, J. (1981). Continuous-genotype models and assortative mating. *Theoretical Population Biol-*  
1171 *ogy*, 19(3):341–357.
- 1172 Fisher, R. A. (1952). Statistical methods in genetics. *Heredity*, 6(1):1–12.
- 1173 Fletcher, J., Wu, Y., Li, T., and Lu, Q. (2021). Interpreting polygenic score effects in sibling analysis.  
1174 *BioRxiv*, doi: <https://doi.org/10.1101/2021.07.16.452740>.
- 1175 Freeman, G. (1973). Statistical methods for the analysis of genotype-environment interactions. *Heredity*,  
1176 31(3):339–354.
- 1177 Fry, A., Littlejohns, T. J., Sudlow, C., Doherty, N., Adamska, L., Sprosen, T., Collins, R., and Allen, N. E.  
1178 (2017). Comparison of sociodemographic and health-related characteristics of UK Biobank participants  
1179 with those of the general population. *American Journal of Epidemiology*, 186(9):1026–1034.

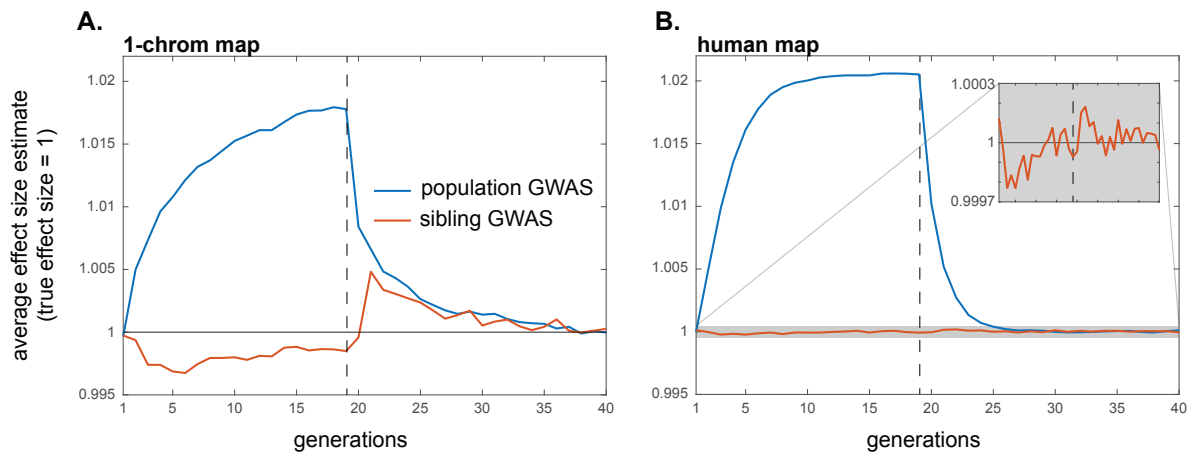
- 1180 Gauderman, W. J., Mukherjee, B., Aschard, H., Hsu, L., Lewinger, J. P., Patel, C. J., Witte, J. S., Amos,  
1181 C., Tai, C. G., Conti, D., et al. (2017). Update on the state of the science for analytical methods for  
1182 gene-environment interactions. *American Journal of Epidemiology*, 186(7):762–770.
- 1183 Greene, W. H. (2018). *Econometric Analysis*. Pearson, New York, 8th edition.
- 1184 Haller, B. C. and Messer, P. W. (2019). SLiM 3: forward genetic simulations beyond the Wright–Fisher  
1185 model. *Molecular Biology and Evolution*, 36(3):632–637.
- 1186 Harpak, A. and Przeworski, M. (2021). The evolution of group differences in changing environments.  
1187 *PLoS Biology*, 19(1):e3001072.
- 1188 Haworth, S., Mitchell, R., Corbin, L., Wade, K. H., Dudding, T., Budu-Aggrey, A., Carslake, D., Hemani,  
1189 G., Paternoster, L., Smith, G. D., et al. (2019). Apparent latent structure within the UK Biobank  
1190 sample has implications for epidemiological analysis. *Nature Communications*, 10(1):1–9.
- 1191 Hayes, B. and Goddard, M. (2010). Genome-wide association and genomic selection in animal breeding.  
1192 *Genome*, 53(11):876–883.
- 1193 Hayward, L. K. and Sella, G. (2022). Polygenic adaptation after a sudden change in environment. *eLife*,  
1194 11:e66697.
- 1195 Horwitz, T. B. and Keller, M. C. (2022). A comprehensive meta-analysis of human assortative mating in  
1196 22 complex traits. *bioRxiv*, doi: <https://doi.org/10.1101/2022.03.19.484997>.
- 1197 Howe, L. J., Nivard, M. G., Morris, T. T., Hansen, A. F., Rasheed, H., Cho, Y., Chittoor, G., Ahlskog,  
1198 R., Lind, P. A., Palviainen, T., et al. (2022). Within-sibship genome-wide association analyses decrease  
1199 bias in estimates of direct genetic effects. *Nature Genetics*, 54(5):581–592.
- 1200 Josephs, E. B., Stinchcombe, J. R., and Wright, S. I. (2017). What can genome-wide association studies tell  
1201 us about the evolutionary forces maintaining genetic variation for quantitative traits? *New Phytologist*,  
1202 214(1):21–33.
- 1203 Kong, A., Thorleifsson, G., Frigge, M. L., Vilhjalmsdottir, B. J., Young, A. I., Thorgeirsson, T. E., Benon-  
1204 isdottir, S., Oddsson, A., Halldorsson, B. V., Masson, G., et al. (2018). The nature of nurture: Effects  
1205 of parental genotypes. *Science*, 359(6374):424–428.
- 1206 Kong, A., Thorleifsson, G., Gudbjartsson, D. F., Masson, G., Sigurdsson, A., Jonasdottir, A., Walters,  
1207 G. B., Jonasdottir, A., Gylfason, A., Kristinsson, K. T., et al. (2010). Fine-scale recombination rate  
1208 differences between sexes, populations and individuals. *Nature*, 467(7319):1099–1103.
- 1209 Lander, E. S. and Schork, N. J. (1994). Genetic dissection of complex traits. *Science*, 265(5181):2037–2048.
- 1210 Lee, H. and Lee, M. H. (2023a). Disentangling linkage and population structure in association mapping.  
1211 [https://github.com/hanbin973/hanbin973.github.io/raw/master/\\_data/LeeAndLee2023a.pdf](https://github.com/hanbin973/hanbin973.github.io/raw/master/_data/LeeAndLee2023a.pdf).
- 1212 Lee, H. and Lee, M. H. (2023b). Theoretical interpretation of genetic studies in admixed populations.  
1213 [https://github.com/hanbin973/hanbin973.github.io/raw/master/\\_data/LeeAndLee2023b.pdf](https://github.com/hanbin973/hanbin973.github.io/raw/master/_data/LeeAndLee2023b.pdf).

- 1214 Lee, J. J., Wedow, R., Okbay, A., Kong, E., Maghzian, O., Zacher, M., Nguyen-Viet, T. A., Bowers,  
1215 P., Sidorenko, J., Karlsson Linnér, R., et al. (2018). Gene discovery and polygenic prediction from a  
1216 genome-wide association study of educational attainment in 1.1 million individuals. *Nature Genetics*,  
1217 50(8):1112–1121.
- 1218 Li, A., Liu, S., Bakshi, A., Jiang, L., Chen, W., Zheng, Z., Sullivan, P. F., Visscher, P. M., Wray, N. R.,  
1219 Yang, J., et al. (2023). mBAT-combo: a more powerful test to detect gene-trait associations from  
1220 GWAS data. *American Journal of Human Genetics*, 110(1):30–43.
- 1221 Marchini, J., Donnelly, P., and Cardon, L. R. (2005). Genome-wide strategies for detecting multiple loci  
1222 that influence complex diseases. *Nature Genetics*, 37(4):413–417.
- 1223 Martin, A. R., Gignoux, C. R., Walters, R. K., Wojcik, G. L., Neale, B. M., Gravel, S., Daly, M. J., Bus-  
1224 tamante, C. D., and Kenny, E. E. (2017). Human demographic history impacts genetic risk prediction  
1225 across diverse populations. *American Journal of Human Genetics*, 100(4):635–649.
- 1226 Morris, T. T., Davies, N. M., Hemani, G., and Smith, G. D. (2020). Population phenomena inflate genetic  
1227 associations of complex social traits. *Science Advances*, 6(16):eaay0328.
- 1228 Mostafavi, H., Harpak, A., Agarwal, I., Conley, D., Pritchard, J. K., and Przeworski, M. (2020). Variable  
1229 prediction accuracy of polygenic scores within an ancestry group. *eLife*, 9:e48376.
- 1230 Nei, M. and Li, W.-H. (1973). Linkage disequilibrium in subdivided populations. *Genetics*, 75(1):213–219.
- 1231 Nivard, M., Belsky, D., Harden, K. P., Baier, T., Ystrom, E., and Lyngstad, T. H. (2022). Neither nature  
1232 nor nurture: Using extended pedigree data to elucidate the origins of indirect genetic effects on offspring  
1233 educational outcomes. *PsyArXiv*, doi: <https://doi.org/10.31234/osf.io/bhpm5>.
- 1234 Okbay, A., Wu, Y., Wang, N., Jayashankar, H., Bennett, M., Nehzati, S. M., Sidorenko, J., Kweon,  
1235 H., Goldman, G., Gjorgjieva, T., et al. (2022). Polygenic prediction of educational attainment within  
1236 and between families from genome-wide association analyses in 3 million individuals. *Nature Genetics*,  
1237 54(4):437–449.
- 1238 Patel, R. A., Musharoff, S. A., Spence, J. P., Pimentel, H., Tcheandjieu, C., Mostafavi, H., Sinnott-  
1239 Armstrong, N., Clarke, S. L., Smith, C. J., Durda, P. P., et al. (2022). Genetic interactions drive  
1240 heterogeneity in causal variant effect sizes for gene expression and complex traits. *American Journal*  
1241 *of Human Genetics*, 109(7):1286–1297.
- 1242 Peiffer, J. A., Romay, M. C., Gore, M. A., Flint-Garcia, S. A., Zhang, Z., Millard, M. J., Gardner, C. A.,  
1243 McMullen, M. D., Holland, J. B., Bradbury, P. J., et al. (2014). The genetic architecture of maize  
1244 height. *Genetics*, 196(4):1337–1356.
- 1245 Pfaff, C. L., Parra, E. J., Bonilla, C., Hiester, K., McKeigue, P. M., Kamboh, M. I., Hutchinson, R. G.,  
1246 Ferrell, R. E., Boerwinkle, E., and Shriver, M. D. (2001). Population structure in admixed populations:  
1247 effect of admixture dynamics on the pattern of linkage disequilibrium. *American Journal of Human*  
1248 *Genetics*, 68(1):198–207.
- 1249 Pirastu, N., Cordioli, M., Nandakumar, P., Mignogna, G., Abdellaoui, A., Hollis, B., Kanai, M., Ra-  
1250 jagopal, V. M., Parolo, P. D. B., Baya, N., et al. (2021). Genetic analyses identify widespread sex-  
1251 differential participation bias. *Nature Genetics*, 53(5):663–671.

- 1252 Platt, A., Vilhjálmsson, B. J., and Nordborg, M. (2010). Conditions under which genome-wide association  
1253 studies will be positively misleading. *Genetics*, 186(3):1045–1052.
- 1254 Price, A. L., Patterson, N. J., Plenge, R. M., Weinblatt, M. E., Shadick, N. A., and Reich, D. (2006).  
1255 Principal components analysis corrects for stratification in genome-wide association studies. *Nature*  
1256 *Genetics*, 38(8):904–909.
- 1257 Price, A. L., Zaitlen, N. A., Reich, D., and Patterson, N. (2010). New approaches to population stratifi-  
1258 cation in genome-wide association studies. *Nature Reviews Genetics*, 11(7):459–463.
- 1259 Pritchard, J. K. and Przeworski, M. (2001). Linkage disequilibrium in humans: models and data. *American*  
1260 *Journal of Human Genetics*, 69(1):1–14.
- 1261 Pritchard, J. K. and Rosenberg, N. A. (1999). Use of unlinked genetic markers to detect population  
1262 stratification in association studies. *American Journal of Human Genetics*, 65(1):220–228.
- 1263 Pritchard, J. K., Stephens, M., Rosenberg, N. A., and Donnelly, P. (2000). Association mapping in  
1264 structured populations. *American Journal of Human Genetics*, 67(1):170–181.
- 1265 Rosenberg, N. A. and Nordborg, M. (2006). A general population-genetic model for the production  
1266 by population structure of spurious genotype–phenotype associations in discrete, admixed or spatially  
1267 distributed populations. *Genetics*, 173(3):1665–1678.
- 1268 Sanjak, J. S., Sidorenko, J., Robinson, M. R., Thornton, K. R., and Visscher, P. M. (2018). Evidence of  
1269 directional and stabilizing selection in contemporary humans. *Proceedings of the National Academy of*  
1270 *Sciences*, 115(1):151–156.
- 1271 Sella, G. and Barton, N. H. (2019). Thinking about the evolution of complex traits in the era of genome-  
1272 wide association studies. *Annual Review of Genomics and Human Genetics*, 20:461–493.
- 1273 Selzam, S., Ritchie, S. J., Pingault, J.-B., Reynolds, C. A., O’Reilly, P. F., and Plomin, R. (2019). Com-  
1274 paring within-and between-family polygenic score prediction. *American Journal of Human Genetics*,  
1275 105(2):351–363.
- 1276 Shen, H. and Feldman, M. W. (2020). Genetic nurturing, missing heritability, and causal analysis in  
1277 genetic statistics. *Proceedings of the National Academy of Sciences*, 117(41):25646–25654.
- 1278 Simons, Y. B., Mostafavi, H., Smith, C. J., Pritchard, J. K., and Sella, G. (2022). Sim-  
1279 ple scaling laws control the genetic architectures of human complex traits. *bioRxiv*, doi:  
1280 <https://doi.org/10.1101/2022.10.04.509926>.
- 1281 Słoczyński, T. (2022). Interpreting OLS estimands when treatment effects are heterogeneous: Smaller  
1282 groups get larger weights. *Review of Economics and Statistics*, 104(3):501–509.
- 1283 Sohail, M., Maier, R. M., Ganna, A., Bloemendal, A., Martin, A. R., Turchin, M. C., Chiang, C. W.,  
1284 Hirschhorn, J., Daly, M. J., Patterson, N., et al. (2019). Polygenic adaptation on height is overestimated  
1285 due to uncorrected stratification in genome-wide association studies. *eLife*, 8:e39702.
- 1286 Spielman, R. S., McGinnis, R. E., and Ewens, W. J. (1993). Transmission test for linkage disequilibrium:  
1287 the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *American Journal of Human*  
1288 *Genetics*, 52(3):506–516.

- 1289 Stulp, G., Simons, M. J. P., Grasman, S., and Pollet, T. V. (2017). Assortative mating for human height:  
1290 A meta-analysis. *American Journal of Human Biology*, 29(1):e22917.
- 1291 Trejo, S. and Domingue, B. W. (2018). Genetic nature or genetic nurture? Introducing social genetic  
1292 parameters to quantify bias in polygenic score analyses. *Biodemography and Social Biology*, 64(3-4):187–  
1293 215.
- 1294 Tropf, F. C., Lee, S. H., Verweij, R. M., Stulp, G., Van Der Most, P. J., De Vlaming, R., Bakshi, A.,  
1295 Briley, D. A., Rahal, C., Hellpap, R., et al. (2017). Hidden heritability due to heterogeneity across  
1296 seven populations. *Nature Human Behaviour*, 1(10):757–765.
- 1297 Tyrrell, J., Zheng, J., Beaumont, R., Hinton, K., Richardson, T. G., Wood, A. R., Davey Smith, G.,  
1298 Frayling, T. M., and Tilling, K. (2021). Genetic predictors of participation in optional components of  
1299 UK Biobank. *Nature Communications*, 12(1):886.
- 1300 Ulizzi, L. and Terrenato, L. (1987). Natural selection associated with birth weight v. the secular relaxation  
1301 of the stabilizing component. *Annals of Human Genetics*, 51(3):205–210.
- 1302 Veller, C., Muralidhar, P., and Haig, D. (2020). On the logic of Fisherian sexual selection. *Evolution*,  
1303 74(7):1234–1245.
- 1304 Vilhjálmsson, B. J. and Nordborg, M. (2013). The nature of confounding in genome-wide association  
1305 studies. *Nature Reviews Genetics*, 14(1):1–2.
- 1306 Visscher, P. M., Medland, S. E., Ferreira, M. A. R., Morley, K. I., Zhu, G., Cornes, B. K., Montgomery,  
1307 G. W., and Martin, N. G. (2006). Assumption-free estimation of heritability from genome-wide identity-  
1308 by-descent sharing between full siblings. *PLoS Genetics*, 2(3):e41.
- 1309 Weiner, D. J., Wigdor, E. M., Ripke, S., Walters, R. K., Kosmicki, J. A., Grove, J., Samocha, K. E.,  
1310 Goldstein, J. I., Okbay, A., Bybjerg-Grauholm, J., et al. (2017). Polygenic transmission disequilibrium  
1311 confirms that common and rare variation act additively to create risk for autism spectrum disorders.  
1312 *Nature Genetics*, 49(7):978–985.
- 1313 Weir, B. S. (2008). Linkage disequilibrium and association mapping. *Annual Review of Genomics and*  
1314 *Human Genetics*, 9(1):129–142.
- 1315 Wolf, J. B., Brodie III, E. D., Cheverud, J. M., Moore, A. J., and Wade, M. J. (1998). Evolutionary  
1316 consequences of indirect genetic effects. *Trends in Ecology & Evolution*, 13(2):64–69.
- 1317 Wright, S. (1921). Systems of mating. III. Assortative mating based on somatic resemblance. *Genetics*,  
1318 6(2):144–161.
- 1319 Yair, S. and Coop, G. (2022). Population differentiation of polygenic score predictions under stabilizing  
1320 selection. *Philosophical Transactions of the Royal Society B*, 377(1852):20200416.
- 1321 Yamamoto, K., Sonehara, K., Namba, S., Konuma, T., Masuko, H., Miyawaki, S., Kamatani, Y., Hizawa,  
1322 N., Ozono, K., Yengo, L., et al. (2023). Genetic footprints of assortative mating in the Japanese  
1323 population. *Nature Human Behaviour*, 7(1):65–73.
- 1324 Yang, J., Zaitlen, N. A., Goddard, M. E., Visscher, P. M., and Price, A. L. (2014). Advantages and  
1325 pitfalls in the application of mixed-model association methods. *Nature Genetics*, 46(2):100–106.

- 1326 Yengo, L., Robinson, M. R., Keller, M. C., Kemper, K. E., Yang, Y., Trzaskowski, M., Gratten, J., Turley,  
1327 P., Cesarini, D., Benjamin, D. J., et al. (2018). Imprint of assortative mating on the human genome.  
1328 *Nature Human Behaviour*, 2(12):948–954.
- 1329 Young, A. I., Benonisdottir, S., Przeworski, M., and Kong, A. (2019). Deconstructing the sources of  
1330 genotype-phenotype associations in humans. *Science*, 365(6460):1396–1400.
- 1331 Young, A. I., Frigge, M. L., Gudbjartsson, D. F., Thorleifsson, G., Bjornsdottir, G., Sulem, P., Masson,  
1332 G., Thorsteinsdottir, U., Stefansson, K., and Kong, A. (2018a). Relatedness disequilibrium regression  
1333 estimates heritability without environmental bias. *Nature Genetics*, 50(9):1304–1310.
- 1334 Young, A. I., Nehzati, S. M., Benonisdottir, S., Okbay, A., Jayashankar, H., Chanwook, L., Cesarini,  
1335 D., Benjamin, D. J., Turley, P., and Kong, A. (2022). Mendelian imputation of parental genotypes  
1336 improves estimates of direct genetic effects. *Nature Genetics*, 54:897–905.
- 1337 Young, A. I., Wauthier, F. L., and Donnelly, P. (2018b). Identifying loci affecting trait variability and  
1338 detecting interactions in genome-wide association studies. *Nature Genetics*, 50(11):1608–1614.
- 1339 Zaidi, A. A. and Mathieson, I. (2020). Demographic history mediates the effect of stratification on  
1340 polygenic scores. *eLife*, 9:e61548.
- 1341 Zaitlen, N., Huntsman, S., Hu, D., Spear, M., Eng, C., Oh, S. S., White, M. J., Mak, A., Davis, A., Meade,  
1342 K., et al. (2017). The effects of migration and assortative mating on admixture linkage disequilibrium.  
1343 *Genetics*, 205(1):375–383.
- 1344 Zaitlen, N., Pasaniuc, B., Sankararaman, S., Bhatia, G., Zhang, J., Gusev, A., Young, T., Tandon, A.,  
1345 Pollack, S., Vilhjálmsson, B. J., et al. (2014). Leveraging population admixture to characterize the  
1346 heritability of complex traits. *Nature Genetics*, 46(12):1356–1362.
- 1347 Zhu, C., Ming, M. J., Cole, J. M., Kirkpatrick, M., and Harpak, A. (2022). Amplifica-  
1348 tion is the primary mode of gene-by-sex interaction in complex human traits. *bioRxiv*, doi:  
1349 <https://doi.org/10.1101/2022.05.06.490973>.



**Figure S1:** Cross-trait assortative mating influences effect size estimates at loci that affect the study trait, although this influence is second-order relative to that on effect size estimates at loci that do not affect the study trait but do affect the other trait involved in assortative mating (note the scale of the y-axis). Simulations are the same as in Fig. 2.



## 1350 **A1 Genetic confounding in population and family-based GWAS de-** 1351 **signs**

### 1352 **A1.1 The model**

1353 Under the general additive model we have studied, an individual's value for trait  $Y$  is

$$1354 \quad Y = Y^* + \sum_{l \in L} \alpha_l^d g_l + \sum_{l \in L} \alpha_l^{i,m} g_l^m + \sum_{l \in L} \alpha_l^{i,f} g_l^f + \epsilon, \quad (\text{A.1})$$

1355

1356 where  $g_l$  is the number of focal alleles at locus  $l$  carried by the individual,  $\alpha_l^d$  is the direct genetic effect  
1357 on the trait value of the focal allele at  $l$  (which we assume to be positive, without loss of generality),  
1358  $g_l^m$  and  $g_l^f$  are the numbers of copies of the focal allele at locus  $l$  carried by the individual's mother and  
1359 father respectively, and  $\alpha_l^{i,m}$  and  $\alpha_l^{i,f}$  are the indirect genetic effects of the focal allele at  $l$  via the mother's  
1360 and father's genotype respectively.  $\epsilon$  is the environmental disturbance, with mean zero, and  $Y^*$  is the  
1361 expected trait value of the offspring of parents who carry only trait-decreasing alleles.

1362 It will be useful to expand Eq. (A.1) in terms of the individual's and the individual's parents' mater-  
1363 nally and paternally inherited genotypes:

$$1364 \quad Y = Y^* + \sum_{l \in L} \alpha_l^d (g_l^{\text{mat}} + g_l^{\text{pat}}) + \sum_{l \in L} \alpha_l^{i,m} (g_l^{\text{m,mat}} + g_l^{\text{m,pat}}) + \sum_{l \in L} \alpha_l^{i,f} (g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) + \epsilon, \quad (\text{A.2})$$

1365 where  $g_l^{\text{mat}}$  is the number of focal alleles at locus  $l$  that the individual inherited maternally,  $g_l^{\text{m,mat}}$  is the  
1366 number of focal alleles at  $l$  that the individual's mother inherited maternally, etc.

### 1367 **A1.2 Population GWAS**

1368 If we perform a standard population GWAS at a genotyped locus  $\lambda$ , the estimated effect of the focal allele  
1369 at  $\lambda$  on the trait  $Y$  is

$$1370 \quad \hat{\alpha}_\lambda^{\text{pop}} = \frac{\text{Cov}(g_\lambda, Y)}{\text{Var}(g_\lambda)}. \quad (\text{A.3})$$

1371 Here,  $\text{Var}(g_\lambda)$  is the genotypic variance at  $\lambda$  among sampled individuals, equal to  $2p_\lambda(1 - p_\lambda)(1 + F_\lambda)$ ,  
1372 where  $p_\lambda$  is the frequency of the focal allele at  $\lambda$  and  $F_\lambda$  is the coefficient of inbreeding at  $\lambda$ . For example,  
1373 if  $\lambda$  is at Hardy-Weinberg equilibrium, then  $\text{Var}(g_\lambda) = 2p_\lambda(1 - p_\lambda)$ ; if, instead, the population is divided  
1374 into several populations, in each of which Hardy-Weinberg equilibrium obtains at  $\lambda$  but between which  
1375 the frequency of the focal variant differs, then  $\text{Var}(g_\lambda) = 2p_\lambda(1 - p_\lambda)(1 + F_{ST,\lambda})$ , where  $F_{ST,\lambda}$  is the value  
1376 of  $F_{ST}$  at locus  $\lambda$ .

1377 The covariance term in Eq. (A.3) expands out to

$$\begin{aligned}
 1378 \quad \text{Cov}(g_\lambda, Y) &= \text{Cov} \left( g_\lambda^{\text{mat}} + g_\lambda^{\text{pat}}, Y^* + \sum_{l \in L} \alpha_l^{\text{d}} (g_l^{\text{mat}} + g_l^{\text{pat}}) \right. \\
 1379 \quad &\quad \left. + \sum_{l \in L} \alpha_l^{\text{i,m}} (g_l^{\text{m,mat}} + g_l^{\text{m,pat}}) + \sum_{l \in L} \alpha_l^{\text{i,f}} (g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) + \epsilon \right) \\
 1380 \quad &= \text{Cov} \left( g_\lambda^{\text{mat}} + g_\lambda^{\text{pat}}, \sum_{l \in L} \alpha_l^{\text{d}} (g_l^{\text{mat}} + g_l^{\text{pat}}) \right) \\
 1381 \quad &\quad + \text{Cov} \left( g_\lambda^{\text{mat}}, \sum_{l \in L} \alpha_l^{\text{i,m}} (g_l^{\text{m,mat}} + g_l^{\text{m,pat}}) + \sum_{l \in L} \alpha_l^{\text{i,f}} (g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) \right) \\
 1382 \quad &\quad + \text{Cov} \left( g_\lambda^{\text{pat}}, \sum_{l \in L} \alpha_l^{\text{i,m}} (g_l^{\text{m,mat}} + g_l^{\text{m,pat}}) + \sum_{l \in L} \alpha_l^{\text{i,f}} (g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) \right) + \text{Cov}(g_\lambda, \epsilon) \\
 1383 \quad &= \sum_{l \in L} \left( \left[ \text{Cov}(g_\lambda^{\text{mat}}, g_l^{\text{mat}}) + \text{Cov}(g_\lambda^{\text{mat}}, g_l^{\text{pat}}) + \text{Cov}(g_\lambda^{\text{pat}}, g_l^{\text{mat}}) + \text{Cov}(g_\lambda^{\text{pat}}, g_l^{\text{pat}}) \right] \alpha_l^{\text{d}} \right. \\
 1384 \quad &\quad \left. + \left[ \text{Cov}(g_\lambda^{\text{mat}}, g_l^{\text{m,mat}} + g_l^{\text{m,pat}}) \right] \alpha_l^{\text{i,m}} + \left[ \text{Cov}(g_\lambda^{\text{pat}}, g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) \right] \alpha_l^{\text{i,f}} \right. \\
 1385 \quad &\quad \left. + \left[ \text{Cov}(g_\lambda^{\text{mat}}, g_l^{\text{f}}) \right] \alpha_l^{\text{i,f}} + \left[ \text{Cov}(g_\lambda^{\text{pat}}, g_l^{\text{m}}) \right] \alpha_l^{\text{i,m}} \right) + \text{Cov}(g_\lambda, \epsilon) \\
 1386 \quad &= \sum_{l \in L} \left( 2 (D_{\lambda l} + \tilde{D}_{\lambda l}) \alpha_l^{\text{d}} \right. \\
 1387 \quad &\quad \left. + \left[ \text{Cov}(g_\lambda^{\text{mat}}, g_l^{\text{m,mat}} + g_l^{\text{m,pat}}) \right] \alpha_l^{\text{i,m}} + \left[ \text{Cov}(g_\lambda^{\text{pat}}, g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) \right] \alpha_l^{\text{i,f}} \right. \\
 1388 \quad &\quad \left. + \left[ \text{Cov}(g_\lambda^{\text{mat}}, g_l^{\text{f}}) \right] \alpha_l^{\text{i,f}} + \left[ \text{Cov}(g_\lambda^{\text{pat}}, g_l^{\text{m}}) \right] \alpha_l^{\text{i,m}} \right) + \text{Cov}(g_\lambda, \epsilon), \tag{A.4} \\
 1389
 \end{aligned}$$

1390 where  $D_{\lambda l}$  and  $\tilde{D}_{\lambda l}$  are the degrees of cis- and trans-linkage disequilibrium between the focal alle-  
 1391 les at loci  $\lambda$  and  $l$  in the GWAS sample. Since  $g_\lambda^{\text{mat}}$  equals  $g_\lambda^{\text{m,mat}}$  or  $g_\lambda^{\text{m,pat}}$  with equal probability,  
 1392  $\text{Cov}(g_\lambda^{\text{mat}}, g_l^{\text{m,mat}} + g_l^{\text{m,pat}}) = D'_{\lambda l} + \tilde{D}'_{\lambda l}$ , and similarly,  $\text{Cov}(g_\lambda^{\text{pat}}, g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) = D'_{\lambda l} + \tilde{D}'_{\lambda l}$  (here,  
 1393  $D'_{\lambda l}$  and  $\tilde{D}'_{\lambda l}$  are the LDs in the parents of the sample, assumed to be equal across mothers and fa-  
 1394 thers). Since maternal transmission is independent of paternal genotype, and vice versa,  $\text{Cov}(g_\lambda^{\text{mat}}, g_l^{\text{f}}) =$   
 1395  $\text{Cov}(g_\lambda^{\text{m}}, g_l^{\text{f}}) / 2$  and  $\text{Cov}(g_\lambda^{\text{pat}}, g_l^{\text{m}}) = \text{Cov}(g_\lambda^{\text{f}}, g_l^{\text{m}}) / 2$ . So

$$\begin{aligned}
 1396 \quad \text{Cov}(g_\lambda, Y) &= \sum_{l \in L} \left( 2 (D_{\lambda l} + \tilde{D}_{\lambda l}) \alpha_l^{\text{d}} + (D'_{\lambda l} + \tilde{D}'_{\lambda l}) (\alpha_l^{\text{i,m}} + \alpha_l^{\text{i,f}}) \right. \\
 1397 \quad &\quad \left. + \frac{1}{2} \left[ \text{Cov}(g_\lambda^{\text{m}}, g_l^{\text{f}}) \right] \alpha_l^{\text{i,f}} + \frac{1}{2} \left[ \text{Cov}(g_\lambda^{\text{f}}, g_l^{\text{m}}) \right] \alpha_l^{\text{i,m}} \right) + \text{Cov}(g_\lambda, \epsilon). \tag{A.5} \\
 1398
 \end{aligned}$$

1399 If  $\alpha_l^{i,m} = \alpha_l^{i,f} = \alpha_l^i$ , then

$$\begin{aligned}
 1400 \text{Cov}(g_\lambda, Y) &= \sum_{l \in L} \left( 2 \left( D_{\lambda l} + \tilde{D}_{\lambda l} \right) \alpha_l^d + \left( 2 \left( D'_{\lambda l} + \tilde{D}'_{\lambda l} \right) + \frac{1}{2} \left[ \text{Cov} \left( g_\lambda^m, g_l^f \right) + \text{Cov} \left( g_\lambda^f, g_l^m \right) \right] \right) \alpha_l^i \right) + \text{Cov}(g_\lambda, \epsilon) \\
 1401 &= \sum_{l \in L} \left( 2 \left( D_{\lambda l} + \tilde{D}_{\lambda l} \right) \alpha_l^d + \left( 2 \left( D'_{\lambda l} + \tilde{D}'_{\lambda l} \right) + \frac{1}{2} \left[ 8\tilde{D}_{\lambda l} \right] \right) \alpha_l^i \right) + \text{Cov}(g_\lambda, \epsilon) \\
 1402 &= 2 \sum_{l \in L} \left( \left( D_{\lambda l} + \tilde{D}_{\lambda l} \right) \alpha_l^d + \left( D'_{\lambda l} + \tilde{D}'_{\lambda l} + 2\tilde{D}_{\lambda l} \right) \alpha_l^i \right) + \text{Cov}(g_\lambda, \epsilon). \tag{A.6} \\
 1403
 \end{aligned}$$

1404 In the second line of Eq. (A.6), we have used the fact that covariances across parents translate to co-  
 1405 variances across maternal and paternal genomes in the offspring. Note, however, that  $\text{Cov} \left( g_\lambda^m, g_l^f \right)$  and  
 1406  $\text{Cov} \left( g_\lambda^f, g_l^m \right)$  need not, in general be equal—e.g., they will not be so under sex-based cross-trait assortative  
 1407 mating—which is why we could not apply a similar simplification to Eq. (A.5).

1408 Dividing Eq. (A.6) by  $\text{Var}(g_\lambda)$ , and recognizing that, for  $l \in L_{\text{local}}$ ,  $c_{\lambda l} \approx 0$ , we recover Eq. (3) in the  
 1409 Main Text.

### 1410 A1.3 Sibling GWAS

1411 Consider two full siblings. Let  $g_l^{\text{mat},1}$  and  $g_l^{\text{mat},2}$  indicate whether sib 1 and sib 2 respectively inherited the  
 1412 focal (trait-increasing) allele from their mother at locus  $l$ . Let  $g_l^{\text{pat},1}$  and  $g_l^{\text{pat},2}$  be analogous indicators  
 1413 for paternal transmission. Write  $\Delta g_l^{\text{mat}} = g_l^{\text{mat},1} - g_l^{\text{mat},2}$  and  $\Delta g_l^{\text{pat}} = g_l^{\text{pat},1} - g_l^{\text{pat},2}$ . Since maternal  
 1414 and paternal transmission are independent,  $\Delta g_l^{\text{mat}}$  and  $\Delta g_{l'}^{\text{pat}}$  are independent for all pairs of loci  $l$  and  $l'$   
 1415 (including  $l = l'$ ). The difference in the two siblings' genotypic values at locus  $l$  is  $\Delta g_l = \Delta g_l^{\text{mat}} + \Delta g_l^{\text{pat}}$ .  
 1416 From Eq. (A.1), the difference in their trait values is

$$1417 \Delta Y = \sum_{l \in L} \Delta g_l \alpha_l^d + \Delta \epsilon, \tag{A.7}$$

1418 where  $\Delta \epsilon$  is the difference in the environmental disturbances experienced by the two siblings. Notice that  
 1419 the indirect effects cancel out of Eq. (A.7), since the parental genotypes are the same for the two siblings.  
 1420 So, in a sib-GWAS for trait  $Y$ , the estimated effect size at  $\lambda$  is

$$\begin{aligned}
 1421 \hat{\alpha}_\lambda^{\text{sib}} &= \frac{\text{Cov}(\Delta g_\lambda, \Delta Y)}{\text{Var}(\Delta g_\lambda)} = \frac{\text{Cov}(\Delta g_\lambda, \sum_{l \in L} \Delta g_l \alpha_l^d + \Delta \epsilon)}{\text{Var}(\Delta g_\lambda)} \\
 1422 &= \frac{\text{Cov}(\Delta g_\lambda^{\text{mat}} + \Delta g_\lambda^{\text{pat}}, \sum_{l \in L} (\Delta g_l^{\text{mat}} + \Delta g_l^{\text{pat}}) \alpha_l^d) + \text{Cov}(\Delta g_\lambda, \Delta \epsilon)}{\text{Var}(\Delta g_\lambda^{\text{mat}} + \Delta g_\lambda^{\text{pat}})} \\
 1423 &= \frac{\sum_{l \in L} \left[ \text{Cov}(\Delta g_\lambda^{\text{mat}}, \Delta g_l^{\text{mat}}) + \text{Cov}(\Delta g_\lambda^{\text{pat}}, \Delta g_l^{\text{pat}}) \right] \alpha_l^d + \text{Cov}(\Delta g_\lambda, \Delta \epsilon)}{\text{Var}(\Delta g_\lambda^{\text{mat}}) + \text{Var}(\Delta g_\lambda^{\text{pat}})} \\
 1424 &= \frac{\sum_{l \in L} \left( \mathbb{E}[\Delta g_\lambda^{\text{mat}} \Delta g_l^{\text{mat}}] + \mathbb{E}[\Delta g_\lambda^{\text{pat}} \Delta g_l^{\text{pat}}] \right) \alpha_l^d + \text{Cov}(\Delta g_\lambda, \Delta \epsilon)}{\mathbb{E}[(\Delta g_\lambda^{\text{mat}})^2] + \mathbb{E}[(\Delta g_\lambda^{\text{pat}})^2]}, \\
 1425
 \end{aligned}$$

1426 since  $\text{Cov}(\Delta g_\lambda^{\text{mat}}, \Delta g_l^{\text{pat}}) = \text{Cov}(\Delta g_l^{\text{mat}}, \Delta g_\lambda^{\text{pat}}) = 0$  (line 3) and  $\mathbb{E}[\Delta g_k^{\text{mat}}] = \mathbb{E}[\Delta g_k^{\text{pat}}] = 0$  for all loci  
 1427  $k$  (line 4). The denominator  $\mathbb{E}[(\Delta g_\lambda^{\text{mat}})^2] + \mathbb{E}[(\Delta g_\lambda^{\text{pat}})^2] = H_\lambda$ , the fraction of parents in the family  
 1428 GWAS sample who are heterozygous at locus  $\lambda$ . The only non-zero contributions to  $\mathbb{E}[\Delta g_\lambda^{\text{mat}} \Delta g_l^{\text{mat}}]$   
 1429 and  $\mathbb{E}[\Delta g_\lambda^{\text{pat}} \Delta g_l^{\text{pat}}]$  come from parents who are heterozygous at both  $\lambda$  and  $l$ . Such parents are either  
 1430 ‘coupling’ double-heterozygotes carrying the focal alleles at  $\lambda$  and  $l$  in coupling phase (i.e., inherited from  
 1431 the same parent), or ‘repulsion’ double-heterozygotes carrying the focal alleles at  $\lambda$  and  $l$  in repulsion  
 1432 phase (inherited from different parents). Among parents, let the fractions of coupling and repulsion  
 1433 double-hets for loci  $\lambda$  and  $l$  be  $H_{\lambda l}^{\text{coup}}$  and  $H_{\lambda l}^{\text{rep}}$  respectively. If the recombination rate between the loci is  
 1434  $c_{\lambda l}^\circ$  in females and  $c_{\lambda l}^\sigma$  in males, then

$$\begin{aligned} \mathbb{E}[\Delta g_\lambda^{\text{mat}} \Delta g_l^{\text{mat}}] &= \mathbb{E}[\Delta g_\lambda^{\text{mat}} \Delta g_l^{\text{mat}} \mid \text{mother is coupling double-het}] H_{\lambda l}^{\text{coup}} \\ &\quad + \mathbb{E}[\Delta g_\lambda^{\text{mat}} \Delta g_l^{\text{mat}} \mid \text{mother is repulsion double-het}] H_{\lambda l}^{\text{rep}} \\ &= \left(\frac{1}{2} - c_{\lambda l}^\circ\right) (H_{\lambda l}^{\text{coup}} - H_{\lambda l}^{\text{rep}}) \\ &= \left(1 - 2c_{\lambda l}^\circ\right) (D'_{\lambda l} - \tilde{D}'_{\lambda l}), \end{aligned}$$

1440 since  $H_{\lambda l}^{\text{coup}} - H_{\lambda l}^{\text{rep}} = 2(D'_{\lambda l} - \tilde{D}'_{\lambda l})$ , where  $D'_{\lambda l}$  and  $\tilde{D}'_{\lambda l}$  are the cis- and trans-LD between the focal/trait-  
 1441 increasing alleles at  $\lambda$  and  $l$  among parents.. Similarly,

$$\mathbb{E}[\Delta g_\lambda^{\text{pat}} \Delta g_l^{\text{pat}}] = \left(1 - 2c_{\lambda l}^\sigma\right) (D'_{\lambda l} - \tilde{D}'_{\lambda l}),$$

1443 So

$$\hat{\alpha}_\lambda^{\text{d,sib}} = \frac{2 \sum_{l \in L} (1 - 2c_{\lambda l}) (D'_{\lambda l} - \tilde{D}'_{\lambda l}) \alpha_l^{\text{d}} + \text{Cov}(\Delta g_l, \Delta \epsilon)}{H_\lambda}, \quad (\text{A.8})$$

1445 where  $c_{\lambda l}$  is the sex-averaged recombination fraction between  $\lambda$  and  $l$ . Since  $\text{Cov}(\Delta g_l, \Delta \epsilon) = 0$ , and  
 1446 recognizing that, for  $l \in L_{\text{local}}$ ,  $c_{\lambda l} \approx 0$  and  $|\tilde{D}'_{\lambda l}| \ll |D'_{\lambda l}|$  in expectation, we recover Eq. (7) in the Main  
 1447 Text.

#### 1448 A1.4 Indirect effects: transmitted vs. untransmitted alleles

1449 In Eq. (A.2),  $g_l^{\text{mat}}$  represents the allele that was transmitted maternally from among the set of maternal  
 1450 alleles  $\{g_l^{\text{m,mat}}, g_l^{\text{m,pat}}\}$ . Thus, if the maternally transmitted allele was the grandmaternal allele (with  
 1451 probability 1/2, and in which case  $g_l^{\text{mat}} = g_l^{\text{m,mat}}$ ), then the untransmitted allele at locus  $l$  is the grandpa-  
 1452 ternal allele, with genotypic value  $g_l^{\text{m,pat}}$ . To make this distinction clear, we write  $g_l^{\text{matT}}$  for the genotypic  
 1453 value of the maternally transmitted allele at locus  $l$ , and  $g_l^{\text{matU}}$  for the maternally untransmitted allele  
 1454 at locus  $l$ . Similarly,  $g_l^{\text{patT}}$  and  $g_l^{\text{patU}}$  represent the paternally transmitted and untransmitted alleles at  $l$ .  
 1455 The transmitted and untransmitted genotypes are  $g_l^{\text{T}} = g_l^{\text{matT}} + g_l^{\text{patT}}$  and  $g_l^{\text{U}} = g_l^{\text{matU}} + g_l^{\text{patU}}$  respectively.

#### 1456 Estimating direct effects

1457 The regressions of the trait value on the transmitted and untransmitted genotypes are

$$\hat{\alpha}_\lambda^{\text{T}} = \frac{\text{Cov}(g_\lambda^{\text{T}}, Y)}{\text{Var}(g_\lambda^{\text{T}})} = \frac{\text{Cov}(g_\lambda^{\text{T}}, Y)}{\text{Var}(g_\lambda)} \quad \text{and} \quad \hat{\alpha}_\lambda^{\text{U}} = \frac{\text{Cov}(g_\lambda^{\text{U}}, Y)}{\text{Var}(g_\lambda^{\text{U}})} = \frac{\text{Cov}(g_\lambda^{\text{U}}, Y)}{\text{Var}(g_\lambda)},$$

1459 where we have used the fact that, since transmission at  $\lambda$  is random,  $\text{Var}(g_\lambda^T) = \text{Var}(g_\lambda^U) = \text{Var}(g_\lambda)$ .

1460 The estimate of the direct effect of the focal variant at  $\lambda$  is then

$$1461 \quad \hat{\alpha}_\lambda^d = \hat{\alpha}_\lambda^T - \hat{\alpha}_\lambda^U = \frac{\text{Cov}(g_\lambda^T, Y) - \text{Cov}(g_\lambda^U, Y)}{\text{Var}(g_\lambda)}.$$

1462 We have

$$1463 \quad \begin{aligned} \text{Cov}(g_\lambda^{\text{matT}}, Y) &= \text{Cov}\left(g_\lambda^{\text{matT}}, Y^* + \sum_{l \in L} (g_l^{\text{matT}} + g_l^{\text{patT}}) \alpha_l^d \right. \\ 1464 &\quad \left. + \sum_{l \in L} (g_l^{\text{m,mat}} + g_l^{\text{m,pat}}) \alpha_l^{\text{i,m}} + \sum_{l \in L} (g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) \alpha_l^{\text{i,f}} + \epsilon\right) \\ 1465 &= \sum_{l \in L} \left[ \text{Cov}(g_\lambda^{\text{matT}}, g_l^{\text{matT}}) + \text{Cov}(g_\lambda^{\text{matT}}, g_l^{\text{patT}}) \right] \alpha_l^d \\ 1466 &\quad + \sum_{l \in L} \left[ \text{Cov}(g_\lambda^{\text{matT}}, g_l^{\text{m,mat}}) + \text{Cov}(g_\lambda^{\text{matT}}, g_l^{\text{m,pat}}) \right] \alpha_l^{\text{i,m}} \\ 1467 &\quad + \sum_{l \in L} \left[ \text{Cov}(g_\lambda^{\text{matT}}, g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) \right] \alpha_l^{\text{i,f}} + \text{Cov}(g_\lambda^{\text{matT}}, \epsilon) \\ 1468 &= \sum_{l \in L} \left[ D'_{\lambda l} (1 - c_{\lambda l}^\circ) + \tilde{D}'_{\lambda l} c_{\lambda l}^\circ + \text{Cov}(g_\lambda^{\text{matT}}, g_l^{\text{patT}}) \right] \alpha_l^d \\ 1469 &\quad + \sum_{l \in L} (D'_{\lambda l} + \tilde{D}'_{\lambda l}) \alpha_l^{\text{i,m}} + \sum_{l \in L} \left[ \text{Cov}(g_\lambda^{\text{matT}}, g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) \right] \alpha_l^{\text{i,f}} + \text{Cov}(g_\lambda^{\text{matT}}, \epsilon), \end{aligned}$$

1471 and

$$1472 \quad \begin{aligned} \text{Cov}(g_\lambda^{\text{matU}}, Y) &= \text{Cov}\left(g_\lambda^{\text{matU}}, Y^* + \sum_{l \in L} (g_l^{\text{matT}} + g_l^{\text{patT}}) \alpha_l^d \right. & (A.9) \\ 1473 &\quad \left. + \sum_{l \in L} (g_l^{\text{m,mat}} + g_l^{\text{m,pat}}) \alpha_l^{\text{i,m}} + \sum_{l \in L} (g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) \alpha_l^{\text{i,f}} + \epsilon\right) \\ 1474 &= \sum_{l \in L} \left[ \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{matT}}) + \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{patT}}) \right] \alpha_l^d \\ 1475 &\quad + \sum_{l \in L} \left[ \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{m,mat}}) + \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{m,pat}}) \right] \alpha_l^{\text{i,m}} \\ 1476 &\quad + \sum_{l \in L} \left[ \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) \right] \alpha_l^{\text{i,f}} + \text{Cov}(g_\lambda^{\text{matU}}, \epsilon) \\ 1477 &= \sum_{l \in L} \left[ D'_{\lambda l} c_{\lambda l}^\circ + \tilde{D}'_{\lambda l} (1 - c_{\lambda l}^\circ) + \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{patT}}) \right] \alpha_l^d \\ 1478 &\quad + \sum_{l \in L} (D'_{\lambda l} + \tilde{D}'_{\lambda l}) \alpha_l^{\text{i,m}} + \sum_{l \in L} \left[ \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) \right] \alpha_l^{\text{i,f}} + \text{Cov}(g_\lambda^{\text{matU}}, \epsilon). \end{aligned}$$

1479 (A.10)

1480 Since  $\text{Cov}(g_\lambda^{\text{matT}}, g_l^{\text{patT}}) = \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{patT}})$  and  $\text{Cov}(g_\lambda^{\text{matT}}, g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) = \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{f,mat}} + g_l^{\text{f,pat}})$ ,

$$1481 \quad \text{Cov}(g_\lambda^{\text{matT}}, Y) - \text{Cov}(g_\lambda^{\text{matU}}, Y) = \sum_{l \in L} \left[ D'_{\lambda l} (1 - c_{\lambda l}^{\circ}) + \tilde{D}'_{\lambda l} c_{\lambda l}^{\circ} \right] \alpha_l^{\text{d}} - \sum_{l \in L} \left[ D'_{\lambda l} c_{\lambda l}^{\circ} + \tilde{D}'_{\lambda l} (1 - c_{\lambda l}^{\circ}) \right] \alpha_l^{\text{d}}$$

$$1482 \quad \quad \quad + \text{Cov}(g_\lambda^{\text{matT}} - g_\lambda^{\text{matU}}, \epsilon)$$

$$1483 \quad \quad \quad = \sum_{l \in L} (1 - 2c_{\lambda l}^{\circ}) (D'_{\lambda l} - \tilde{D}'_{\lambda l}) \alpha_l^{\text{d}} + \text{Cov}(g_\lambda^{\text{matT}} - g_\lambda^{\text{matU}}, \epsilon).$$

1485 Similarly,

$$1486 \quad \text{Cov}(g_\lambda^{\text{patT}}, Y) - \text{Cov}(g_\lambda^{\text{patU}}, Y) = \sum_{l \in L} (1 - 2c_{\lambda l}^{\sigma}) (D'_{\lambda l} - \tilde{D}'_{\lambda l}) \alpha_l^{\text{d}} + \text{Cov}(g_\lambda^{\text{patT}} - g_\lambda^{\text{patU}}, \epsilon).$$

1487 Since  $g_\lambda^{\text{T}} = g_\lambda^{\text{matT}} + g_\lambda^{\text{patT}}$  and  $g_\lambda^{\text{U}} = g_\lambda^{\text{matU}} + g_\lambda^{\text{patU}}$ ,

$$1488 \quad \text{Cov}(g_\lambda^{\text{T}}, Y) - \text{Cov}(g_\lambda^{\text{U}}, Y) = 2 \sum_{l \in L} (1 - 2c_{\lambda l}) (D'_{\lambda l} - \tilde{D}'_{\lambda l}) \alpha_l^{\text{d}} + \text{Cov}(g_\lambda^{\text{T}} - g_\lambda^{\text{U}}, \epsilon),$$

1489 where  $c_{\lambda l}$  is the sex-averaged recombination fraction between  $\lambda$  and  $l$ . Therefore, the transmitted-  
1490 untransmitted regression coefficient at locus  $\lambda$  is

$$1491 \quad \hat{\alpha}_\lambda^{\text{d,T-U}} = \frac{\text{Cov}(g_\lambda^{\text{T}}, Y) - \text{Cov}(g_\lambda^{\text{U}}, Y)}{\text{Var}(g_\lambda)} = \frac{2 \sum_{l \in L} (1 - 2c_{\lambda l}) (D'_{\lambda l} - \tilde{D}'_{\lambda l}) \alpha_l^{\text{d}} + \text{Cov}(g_\lambda^{\text{T}} - g_\lambda^{\text{U}}, \epsilon)}{V_\lambda}. \quad (\text{A.11})$$

## 1492 Estimating indirect effects

1493 The coefficient in the regression of the trait value  $Y$  on the untransmitted genotype  $g_\lambda^{\text{U}}$  at locus  $\lambda$ ,  $\hat{\alpha}_\lambda^{\text{U}}$ ,  
1494 has sometimes been considered to provide an estimate of the indirect ‘family’ effect of the focal variant  
1495 at  $\lambda$ :  $\hat{\alpha}_\lambda^{\text{i}} = \hat{\alpha}_\lambda^{\text{U}}$ . From Eq. (A.10) and its analog for the paternally untransmitted allele,

$$1496 \quad \text{Cov}(g_\lambda^{\text{matU}} + g_\lambda^{\text{patU}}, Y) = \sum_{l \in L} \left( \left[ 2D'_{\lambda l} c_{\lambda l} + 2\tilde{D}'_{\lambda l} (1 - c_{\lambda l}) + \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{patT}}) + \text{Cov}(g_\lambda^{\text{patU}}, g_l^{\text{matT}}) \right] \alpha_l^{\text{d}} \right.$$

$$1497 \quad \quad \quad \left. + (D'_{\lambda l} + \tilde{D}'_{\lambda l}) \alpha_l^{\text{i,m}} + \sum_{l \in L} \left[ \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) \right] \alpha_l^{\text{i,f}} \right.$$

$$1498 \quad \quad \quad \left. + (D'_{\lambda l} + \tilde{D}'_{\lambda l}) \alpha_l^{\text{i,f}} + \sum_{l \in L} \left[ \text{Cov}(g_\lambda^{\text{patU}}, g_l^{\text{m,mat}} + g_l^{\text{m,pat}}) \right] \alpha_l^{\text{i,m}} \right)$$

$$1499 \quad \quad \quad + \text{Cov}(g_\lambda^{\text{matU}}, \epsilon) + \text{Cov}(g_\lambda^{\text{patU}}, \epsilon),$$

1501 where  $c_{\lambda l}$  is the sex-averaged recombination fraction. In this expression,

$$1502 \quad \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{patT}}) + \text{Cov}(g_\lambda^{\text{patU}}, g_l^{\text{matT}}) = \text{Cov}(g_\lambda^{\text{matT}}, g_l^{\text{patT}}) + \text{Cov}(g_\lambda^{\text{patT}}, g_l^{\text{matT}}) = 2\tilde{D}_{\lambda l},$$

1503 while

$$1504 \quad \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{f,mat}} + g_l^{\text{f,pat}}) = \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{patU}} + g_l^{\text{patT}}) = \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{patU}}) + \text{Cov}(g_\lambda^{\text{matU}}, g_l^{\text{patT}}) = 2\tilde{D}_{\lambda l},$$

1505 and, similarly,  $\text{Cov}\left(g_\lambda^{\text{patU}}, g_l^{\text{m,mat}} + g_l^{\text{m,pat}}\right) = 2\tilde{D}_{\lambda l}$ . So

$$1506 \quad \text{Cov}\left(g_\lambda^{\text{U}}, Y\right) = \text{Cov}\left(g_\lambda^{\text{matU}} + g_\lambda^{\text{patU}}, Y\right) = \sum_{l \in L} \left[ 2\left(D'_{\lambda l}c_{\lambda l} + \tilde{D}'_{\lambda l}(1 - c_{\lambda l}) + \tilde{D}_{\lambda l}\right) \alpha_l^{\text{d}} \right. \\ 1507 \quad \left. + \left(D'_{\lambda l} + \tilde{D}'_{\lambda l} + 2\tilde{D}_{\lambda l}\right) \left(\alpha_l^{\text{i,m}} + \alpha_l^{\text{i,f}}\right) \right] + \text{Cov}\left(g_\lambda^{\text{U}}, \epsilon\right). \quad (\text{A.12})$$

1509 If we assume that indirect effects via the maternal and paternal families are equal ( $\alpha_l^{\text{i,m}} = \alpha_l^{\text{i,f}} = \alpha_l^{\text{i}}$ ),  
1510 then Eq. (A.12) simplifies further to

$$1511 \quad \text{Cov}\left(g_\lambda^{\text{U}}, Y\right) = 2 \sum_{l \in L} \left[ \left(D'_{\lambda l}c_{\lambda l} + \tilde{D}'_{\lambda l}(1 - c_{\lambda l}) + \tilde{D}_{\lambda l}\right) \alpha_l^{\text{d}} + \left(D'_{\lambda l} + \tilde{D}'_{\lambda l} + 2\tilde{D}_{\lambda l}\right) \alpha_l^{\text{i}} \right] + \text{Cov}\left(g_\lambda^{\text{U}}, \epsilon\right). \quad (\text{A.13})$$

1512 In this case, the estimate of the indirect effect of the focal allele at  $\lambda$  is

$$1513 \quad \hat{\alpha}_\lambda^{\text{i}} = \frac{\text{Cov}\left(g_\lambda^{\text{U}}, Y\right)}{\text{Var}\left(g_\lambda\right)} = \frac{2 \sum_{l \in L} \left[ \left(D'_{\lambda l}c_{\lambda l} + \tilde{D}'_{\lambda l}(1 - c_{\lambda l}) + \tilde{D}_{\lambda l}\right) \alpha_l^{\text{d}} + \left(D'_{\lambda l} + \tilde{D}'_{\lambda l} + 2\tilde{D}_{\lambda l}\right) \alpha_l^{\text{i}} \right] + \text{Cov}\left(g_\lambda^{\text{U}}, \epsilon\right)}{\text{Var}\left(g_\lambda\right)}. \quad (\text{A.14})$$

## 1514 A2 Polygenic scores and their phenotypic correlations

1515 Suppose that we have estimated effect sizes  $\hat{\alpha}_\lambda$  at a set of genotyped loci  $\lambda \in \Lambda$  using a population GWAS  
1516 for trait 1. For each individual, we can then compute a polygenic score:

$$1517 \quad PGS_1 = \sum_{\lambda \in \Lambda} g_\lambda \hat{\alpha}_\lambda^{\text{pop}}. \quad (\text{A.15})$$

1518 PGSs are often treated as predictions of individuals' genetic values for traits. In this regard, we might  
1519 therefore be interested in the covariance across the population between the PGS for a trait and individuals'  
1520 values for that trait:  $\text{Cov}(PGS_1, Y_1)$ . Additionally, if PGSs are treated as predictions of genetic values  
1521 of traits, then we might be interested in how the PGS calculated for one trait covaries with the value  
1522 of another trait:  $\text{Cov}(PGS_1, Y_2)$ . Such covariances might be informative of genetic correlations between  
1523 traits, or pleiotropy of the alleles underlying genetic variation in the traits. We focus on the two-trait  
1524 covariance, since it nests the single-trait covariance as a special case. If the total set of loci causally  
1525 underlying variation in traits 1 and 2 is  $L$ , then the population covariance between the PGS for trait 1  
1526 and the value of trait 2 is

$$1527 \quad \text{Cov}\left(PGS_1, Y_2\right) = \text{Cov}\left(\sum_{\lambda \in \Lambda} g_\lambda \hat{\alpha}_\lambda^{\text{pop}}, \sum_{l \in L} g_l \beta_l\right) \\ 1528 \quad = \text{Cov}\left(\sum_{\lambda \in \Lambda} (g_\lambda^{\text{m}} + g_\lambda^{\text{p}}) \hat{\alpha}_\lambda^{\text{pop}}, \sum_{l \in L} (g_l^{\text{m}} + g_l^{\text{p}}) \beta_l\right) \\ 1529 \quad = 2 \sum_{\lambda \in \Lambda} \sum_{l \in L} \left(D_{\lambda l} + \tilde{D}_{\lambda l}\right) \hat{\alpha}_\lambda^{\text{pop}} \beta_l. \quad (\text{A.16})$$

1531 The effect size estimates from the population GWAS for trait 1 are

$$1532 \quad \hat{\alpha}_\lambda^{\text{pop}} = \frac{2}{V_\lambda} \sum_{l' \in L} (D_{\lambda l'} + \tilde{D}_{\lambda l'}) \alpha_{l'} \approx \alpha_\lambda + \frac{2}{V_\lambda} \sum_{\substack{l' \in L \\ l' \neq \lambda}} (D_{\lambda l'} + \tilde{D}_{\lambda l'}) \alpha_{l'},$$

1533 and so Eq. (A.16) is, in general,

$$1534 \quad \text{Cov}(PGS_1, Y_2) = \sum_{\lambda \in \Lambda} 2p_\lambda(1-p_\lambda)\alpha_\lambda\beta_\lambda + 2 \sum_{\lambda \in \Lambda} \sum_{\substack{l \in L \\ l \neq \lambda}} (D_{\lambda l} + \tilde{D}_{\lambda l}) \alpha_\lambda \beta_l \quad (\text{A.17})$$

$$1535 \quad + 4 \sum_{\lambda \in \Lambda} \sum_{\substack{l' \in L \\ l' \neq \lambda}} \sum_{\substack{l \in L \\ l \neq \lambda}} \frac{1}{V_\lambda} (D_{\lambda l'} + \tilde{D}_{\lambda l'}) (D_{\lambda l} + \tilde{D}_{\lambda l}) \alpha_{l'} \beta_l. \quad (\text{A.18})$$

1537 In a family-based study, we might instead be interested in the covariance between siblings' differences  
1538 in the trait-1 population PGS and their differences in trait 2. We can write this covariance in our model  
1539 as

$$1540 \quad \text{Cov}(\Delta PGS_1, \Delta Y_2) = \text{Cov} \left( \sum_{\lambda \in \Lambda} (\Delta g_\lambda^m + \Delta g_\lambda^p) \hat{\alpha}_\lambda^{\text{POP}}, \sum_{l \in L} (\Delta g_l^m + \Delta g_l^p) \beta_l \right)$$

$$1541 \quad = \mathbb{E} \left[ \left( \sum_{\lambda \in \Lambda} (\Delta g_\lambda^m + \Delta g_\lambda^p) \hat{\alpha}_\lambda^{\text{POP}} \right) \left( \sum_{l \in L} (\Delta g_l^m + \Delta g_l^p) \beta_l \right) \right]$$

$$1542 \quad = \sum_{\lambda \in \Lambda} \sum_{l \in L} \mathbb{E} [(\Delta g_\lambda^m + \Delta g_\lambda^p) (\Delta g_l^m + \Delta g_l^p) \hat{\alpha}_\lambda^{\text{POP}} \beta_l]$$

$$1543 \quad = \sum_{\lambda \in \Lambda} \sum_{l \in L} (\mathbb{E} [\Delta g_\lambda^m \Delta g_l^m \hat{\alpha}_\lambda^{\text{POP}} \beta_l] + \mathbb{E} [\Delta g_\lambda^p \Delta g_l^p \hat{\alpha}_\lambda^{\text{POP}} \beta_l]), \quad (\text{A.19})$$

1545 since maternal and paternal transmission are conditionally independent. Focusing on maternal transmis-  
1546 sion, and writing  $h_{\lambda l}^{c,m}$  and  $h_{\lambda l}^{r,m}$  for the events that the mother is respectively a coupling and a repulsion  
1547 heterozygote at loci  $\lambda$  and  $l$ , with  $H_{\lambda l}^{\text{coup}}$  and  $H_{\lambda l}^{\text{rep}}$  their associated probabilities (which are assumed to  
1548 be the same for mothers and fathers),

$$1549 \quad \mathbb{E} [\Delta g_\lambda^m \Delta g_l^m \hat{\alpha}_\lambda^{\text{POP}} \beta_l] = \mathbb{E} [\Delta g_\lambda^m \Delta g_l^m \hat{\alpha}_\lambda^{\text{POP}} \beta_l | h_{\lambda l}^{c,m}] H_{\lambda l}^{\text{coup}} + \mathbb{E} [\Delta g_\lambda^m \Delta g_l^m \hat{\alpha}_\lambda^{\text{POP}} \beta_l | h_{\lambda l}^{r,m}] H_{\lambda l}^{\text{rep}}$$

$$1550 \quad = (\mathbb{E} [\Delta g_\lambda^m \Delta g_l^m | h_{\lambda l}^{c,m}] H_{\lambda l}^{\text{coup}} + \mathbb{E} [\Delta g_\lambda^m \Delta g_l^m | h_{\lambda l}^{r,m}] H_{\lambda l}^{\text{rep}}) \hat{\alpha}_\lambda^{\text{POP}} \beta_l$$

$$1551 \quad = \left( \frac{1}{2} - c_{\lambda l}^\circ \right) (H_{\lambda l}^{\text{coup}} - H_{\lambda l}^{\text{rep}}) \hat{\alpha}_\lambda^{\text{POP}} \beta_l$$

$$1552 \quad = (1 - 2c_{\lambda l}^\circ) (D'_{\lambda l} - \tilde{D}'_{\lambda l}) \hat{\alpha}_\lambda^{\text{POP}} \beta_l,$$

1554 with  $D'_{\lambda l}$  and  $\tilde{D}'_{\lambda l}$  measured in the parents. Similarly,

$$1555 \quad \mathbb{E} [\Delta g_\lambda^p \Delta g_l^p \hat{\alpha}_\lambda^{\text{POP}} \beta_l] = (1 - 2c_{\lambda l}^\sigma) (D'_{\lambda l} - \tilde{D}'_{\lambda l}) \hat{\alpha}_\lambda^{\text{POP}} \beta_l,$$

1556 and so Eq. (A.19) becomes

$$1557 \quad \text{Cov}(\Delta PGS_1, \Delta Y_2) = 2 \sum_{\lambda \in \Lambda} \sum_{l \in L} (1 - 2c_{\lambda l}) (D'_{\lambda l} - \tilde{D}'_{\lambda l}) \hat{\alpha}_\lambda^{\text{POP}} \beta_l, \quad (\text{A.20})$$

1558 where  $c_{\lambda l}$  is the sex-averaged recombination fraction between  $\lambda$  and  $l$ .

1559 Before we substitute the population GWAS estimates  $\hat{\alpha}_\lambda^{\text{POP}}$  into Eq. (A.20), it is worth considering what  
1560 value this expression would take if effect sizes were correctly estimated at every study locus,  $\hat{\alpha}_\lambda^{\text{POP}} = \alpha_\lambda$ .



1561 In this case, Eq. (A.20) becomes

$$\begin{aligned}
 1562 \quad \text{Cov}(\Delta PGS_1, \Delta Y_2) &= 2 \sum_{\lambda \in \Lambda} \sum_{l \in L} (1 - 2c_{\lambda l}) \left( D_{\lambda l} - \tilde{D}_{\lambda l} \right) \alpha_{\lambda} \beta_l \\
 1563 \quad &= \sum_{\lambda \in \Lambda} 2p_{\lambda}(1 - p_{\lambda}) \alpha_{\lambda} \beta_{\lambda} + 2 \sum_{\lambda \in \Lambda} \sum_{\substack{l \in L \\ l \neq \lambda}} (1 - 2c_{\lambda l}) \left( D'_{\lambda l} - \tilde{D}'_{\lambda l} \right) \alpha_{\lambda} \beta_l. \quad (\text{A.21})
 \end{aligned}$$

1564

1565 If the two traits are distinct, then the first term in Eq. (A.21) is the genic covariance of traits 1 and  
 1566 2 across the set of study loci (more precisely, tagged locally by the study loci), and reflects systematic  
 1567 pleiotropy at these loci; this term would, for example, be positive if alleles tend to have same-direction  
 1568 effects on traits 1 and 2. If we were studying only one trait, then  $\alpha_{\lambda} = \beta_{\lambda}$ , and the first term would be  
 1569 the genic variance of the trait across study loci,  $\sum_{\lambda \in \Lambda} 2p_{\lambda}(1 - p_{\lambda}) \alpha_{\lambda}^2$ . The second term in Eq. (A.21) is  
 1570 an effect of linkage disequilibria between study loci and the loci that are causal for trait 2; these LDs are  
 1571 absorbed by the PGS because the PGS is a sum across loci. In the absence of such LDs, or in cases where  
 1572 the cis- and trans-LDs are equal so that  $D'_{\lambda l} - \tilde{D}'_{\lambda l} = 0$ , Eq. (A.21) would equal the genic variance in the  
 1573 single-trait case and the genic covariance in the two-trait case.

1574 The effect size estimates from a population GWAS are in fact

$$1575 \quad \hat{\alpha}_{\lambda}^{\text{POP}} = \frac{2}{V_{\lambda}} \sum_{l' \in L} (D_{\lambda l'} + \tilde{D}_{\lambda l'}) \alpha_{l'} \approx \alpha_{\lambda} + \frac{2}{V_{\lambda}} \sum_{\substack{l' \in L \\ l' \neq \lambda}} (D_{\lambda l'} + \tilde{D}_{\lambda l'}) \alpha_{l'},$$

1576  $D_{\lambda l'}$  and  $\tilde{D}_{\lambda l'}$  are measured in the sample. We assume these to be equal to the values in parents in the  
 1577 family-based GWAS,  $D'_{\lambda l}$  and  $\tilde{D}'_{\lambda l}$ , and so the value taken by Eq. (A.20) is

$$\begin{aligned}
 1578 \quad \text{Cov}(\Delta PGS_1, \Delta Y_2) &= 2 \sum_{\lambda \in \Lambda} \sum_{l \in L} (1 - 2c_{\lambda l}) \left( D_{\lambda l} - \tilde{D}_{\lambda l} \right) \hat{\alpha}_{\lambda}^{\text{POP}} \beta_l \\
 1579 \quad &= 2 \sum_{\lambda \in \Lambda} \sum_{l \in L} (1 - 2c_{\lambda l}) \left( D_{\lambda l} - \tilde{D}_{\lambda l} \right) \left( \alpha_{\lambda} + \frac{2}{V_{\lambda}} \sum_{\substack{l' \in L \\ l' \neq \lambda}} (D_{\lambda l'} + \tilde{D}_{\lambda l'}) \alpha_{l'} \right) \beta_l \\
 1580 \quad &= \underbrace{\sum_{\lambda \in \Lambda} 2p_{\lambda}(1 - p_{\lambda}) \alpha_{\lambda} \beta_{\lambda}}_{\text{pleiotropy}} + \underbrace{2 \sum_{\lambda \in \Lambda} \sum_{\substack{l \in L \\ l \neq \lambda}} (1 - 2c_{\lambda l}) \left( D_{\lambda l} - \tilde{D}_{\lambda l} \right) \alpha_{\lambda} \beta_l}_{\text{covariance from LD absorbed by PGS because it is a sum across loci}} \\
 1581 \quad &\quad + \underbrace{4 \sum_{\lambda \in \Lambda} \sum_{\substack{l \in L \\ l \neq \lambda}} (1 - 2c_{\lambda l}) \left( D_{\lambda l}^2 - \tilde{D}_{\lambda l}^2 \right) \alpha_l \beta_l / V_{\lambda}}_{\text{covariance from LD absorbed by PGS because effect size estimates absorb LD}} \\
 1582 \quad &\quad + \underbrace{4 \sum_{\lambda \in \Lambda} \sum_{\substack{l \in L \\ l \neq \lambda}} \sum_{\substack{l' \in L \\ l' \neq \lambda, l}} (1 - 2c_{\lambda l}) \left( D_{\lambda l'} + \tilde{D}_{\lambda l'} \right) \alpha_{l'} \left( D_{\lambda l} - \tilde{D}_{\lambda l} \right) \beta_l / V_{\lambda}}_{\text{covariance from systematic LD between variants with same directional effect on trait}}. \quad (\text{A.22})
 \end{aligned}$$

1583

1584 In the absence of genetic confounding ( $D_{\lambda l} = \tilde{D}_{\lambda l} = 0$ ) or, more generally, if genetic stratification is such  
 1585 that the cis- and trans-LDs are equal ( $D_{\lambda l} - \tilde{D}_{\lambda l} = 0$ ), then Eq. (A.22) simplifies to the SNP-tagged genic

1586 covariance between traits 1 and 2:

$$1587 \quad \text{Cov}(\Delta PGS_1, \Delta Y_2) = \sum_{\lambda \in \Lambda} 2p_\lambda(1 - p_\lambda)\alpha_\lambda\beta_\lambda. \quad (\text{A.23})$$

1588 If traits 1 and 2 are the same, then this is simply the SNP-tagged genic variance of the trait:  $\text{Cov}(\Delta PGS, \Delta Y) =$   
 1589  $\sum_{\lambda \in \Lambda} 2p_\lambda(1 - p_\lambda)\alpha_\lambda^2$ .

1590 Eq. (A.22) simplifies somewhat if we focus on a single trait ( $\alpha_l = \beta_l$ ) and assume that there is no  
 1591 trans-LD ( $\tilde{D}_{\lambda l} = 0$ ); in this case,

$$1592 \quad \text{Cov}(\Delta PGS, \Delta Y) = \underbrace{\sum_{\lambda \in \Lambda} 2p_\lambda(1 - p_\lambda)\alpha_\lambda^2}_{\text{SNP-tagged genic variance}} + \underbrace{2 \sum_{\lambda \in \Lambda} \sum_{\substack{l \in L \\ l \neq \lambda}} (1 - 2c_{\lambda l})D_{\lambda l}\alpha_\lambda\alpha_l}_{\text{variance from LD absorbed by PGS because it is a sum across loci}}$$

$$1593 \quad + \underbrace{4 \sum_{\lambda \in \Lambda} \sum_{\substack{l \in L \\ l \neq \lambda}} (1 - 2c_{\lambda l})D_{\lambda l}^2\alpha_l^2/V_\lambda}_{\text{variance from LD absorbed by PGS because effect size estimates absorb LD}} + \underbrace{4 \sum_{\lambda \in \Lambda} \sum_{\substack{l \in L \\ l \neq \lambda}} \sum_{\substack{l' \in L \\ l' \neq \lambda, l}} (1 - 2c_{\lambda l})D_{\lambda l}\alpha_l D_{\lambda l'}\alpha_{l'}/V_\lambda}_{\text{variance from systematic LD between variants with same directional effect on trait}}.$$

1594 (A.24)

## 1595 A3 Sources of genetic confounding

1596 The calculations above reveal that genetic confounds in GWAS designs can depend on long-range LD in  
 1597 the sample and among parents of the sample. Here, we consider several possible sources of long-range  
 1598 LD.

### 1599 A3.1 Assortative mating

1600 If there is a constant correlation among mates for their values of two traits, then a genetic equilibrium  
 1601 will eventually be achieved. In this equilibrium, for any pair of loci  $l$  and  $l'$ , the trans-LD  $\tilde{D}_{ll'}$  will be  
 1602 constant. Call this constant value  $D_{ll'}^*$ , and suppose that the recombination fraction between the loci  
 1603 is  $c_{ll'}$ . With  $\tilde{D}_{ll'}$  constant across generations, the balance of its conversion into cis-LD (at rate  $c_{ll'}$  per  
 1604 generation) and the destruction of cis-LD by recombination (at rate  $c_{ll'}$  per generation) will result in an  
 1605 equilibrium level of cis-LD equal to the degree of trans-LD:  $D_{ll'} = \tilde{D}_{ll'} = D_{ll'}^*$  (e.g., Crow and Felsenstein  
 1606 1968).

1607 The value of  $D_{ll'}^*$  will, in general, depend in a complicated way on the strength of effects of  $l$  and  $l'$  on  
 1608 the traits upon which assortative mating is based and on the linkage relations of these loci to one another  
 1609 and to other causal loci. However, while it is therefore difficult to calculate the individual equilibrium LD  
 1610 terms  $D_{ll'}^*$ , we can in some cases calculate weighted sums of these terms across locus pairs.

1611 Let the set of loci that influence one or both traits be  $L$ , and let  $\alpha_l$  be the effect size of the focal variant  
 1612 at locus  $l$  on trait 1 and  $\beta_l$  its effect on trait 2 (the analyses below also apply to same-trait assortative  
 1613 mating, setting  $\alpha_l = \beta_l$ ). Recall the notation  $g_l^{\text{m,mat}}$  and  $g_l^{\text{m,pat}}$  for a mother's maternally and paternally  
 1614 inherited genotype at locus  $l$ , with  $g_l^{\text{f,mat}}$  and  $g_l^{\text{f,pat}}$  a father's analogs. The mother's breeding value for  
 1615 trait 1 is

$$1616 \quad G_1^{\text{m}} = \sum_{l \in L} g_l^{\text{m}} \alpha_l = \sum_{l \in L} (g_l^{\text{m,mat}} + g_l^{\text{m,pat}}) \alpha_l = \sum_{l \in L} g_l^{\text{m,mat}} \alpha_l + \sum_{l \in L} g_l^{\text{m,pat}} \alpha_l = G_1^{\text{m,mat}} + G_1^{\text{m,pat}},$$

1617 and, similarly, her breeding value for trait 2 is

$$1618 \quad G_2^m = \sum_{l \in L} g_l^{m, \text{mat}} \beta_l + \sum_{l \in L} g_l^{m, \text{pat}} \beta_l = G_2^{m, \text{mat}} + G_2^{m, \text{pat}}.$$

1619 The father's breeding values for the two traits are

$$1620 \quad G_1^f = \sum_{l \in L} g_l^{f, \text{mat}} \alpha_l + \sum_{l \in L} g_l^{f, \text{pat}} \alpha_l = G_1^{f, \text{mat}} + G_1^{f, \text{pat}}$$

1621 and

$$1622 \quad G_2^f = \sum_{l \in L} g_l^{f, \text{mat}} \beta_l + \sum_{l \in L} g_l^{f, \text{pat}} \beta_l = G_2^{f, \text{mat}} + G_2^{f, \text{pat}}.$$

1623 We assume that individual trait values equal the breeding values plus environmental disturbances that  
1624 are uncorrelated with the breeding values:

$$1625 \quad Y_1^m = G_1^m + \epsilon_1^m; \quad Y_2^m = G_2^m + \epsilon_2^m; \quad Y_1^f = G_1^f + \epsilon_1^f; \quad Y_2^f = G_2^f + \epsilon_2^f;$$

1626 where

$$1627 \quad \text{Var}(\epsilon_1^m) = \text{Var}(\epsilon_1^f) = V_E^1, \quad \text{Var}(\epsilon_2^m) = \text{Var}(\epsilon_2^f) = V_E^2,$$

1628 and

$$1629 \quad \text{Cov}(\epsilon_i^m, G_i^m) = \text{Cov}(\epsilon_i^f, G_i^f) = 0 \text{ for } i \in \{1, 2\}.$$

### 1630 **A3.1.1 Same-trait assortative mating, or cross-trait assortative mating that is symmetric** 1631 **with respect to sex**

1632 We first consider the case where the strength of assortative mating between two traits, as measured by  
1633 their correlation coefficient across mating pairs, is equal in the female-male and male-female directions.  
1634 Notice that this scenario covers same-trait assortative mating. In the case of cross-trait assortative  
1635 mating, it could occur if assortative mating arises by mechanisms other than direct female (or male)  
1636 mating preferences.

1637 We assume that there is a constant correlation  $\rho$  among mating pairs for their phenotypic values of  
1638 traits 1 and 2. In equilibrium, this will translate to a constant correlation  $\rho_G$  between their breeding  
1639 values as well (e.g., Felsenstein 1981). To calculate  $\rho_G$ , we first note that, because assortative mating is  
1640 based on phenotypic values and not breeding values per se, if we know the phenotypes of a pair of mates,  
1641 we obtain no further information about the similarity of their breeding values; that is,

$$1642 \quad \text{Cov} \left( G_1^m, G_2^f \mid \left\{ Y_1^m, Y_2^f \right\} \right) = \text{Cov} \left( G_2^m, G_1^f \mid \left\{ Y_2^m, Y_1^f \right\} \right) = 0. \quad (\text{A.25})$$

1643 For the same reason, if we know the phenotypic values of two mates, then the trait-2 value of the male  
1644 does not offer any information on the female's trait-1 breeding value beyond that already offered by the  
1645 female's trait-1 phenotype, and vice versa; that is,

$$1646 \quad \begin{aligned} \mathbb{E} \left[ G_1^m \mid \left\{ Y_1^m, Y_2^f \right\} \right] &= \mathbb{E} \left[ G_1^m \mid Y_1^m \right]; & \mathbb{E} \left[ G_2^f \mid \left\{ Y_1^m, Y_2^f \right\} \right] &= \mathbb{E} \left[ G_2^f \mid Y_2^f \right]; \\ \mathbb{E} \left[ G_2^m \mid \left\{ Y_2^m, Y_1^f \right\} \right] &= \mathbb{E} \left[ G_2^m \mid Y_2^m \right]; & \mathbb{E} \left[ G_1^f \mid \left\{ Y_2^m, Y_1^f \right\} \right] &= \mathbb{E} \left[ G_1^f \mid Y_1^f \right]. \end{aligned} \quad (\text{A.26})$$

1649 If  $Y_1$  and  $G_1$ , and similarly  $Y_2$  and  $G_2$ , are bivariate normal, then

$$1650 \quad \mathbb{E}[G_1 | Y_1] = \mathbb{E}[G_1] + h_1^2(Y_1 - \mathbb{E}[Y_1]) \quad \text{and} \quad \mathbb{E}[G_2 | Y_2] = \mathbb{E}[G_2] + h_2^2(Y_2 - \mathbb{E}[Y_2]) \quad (\text{A.27})$$

1651 where  $h_1^2$  and  $h_2^2$  are the heritabilities of traits 1 and 2, respectively.

1652 From the law of total covariance,

$$1653 \quad \begin{aligned} \text{Cov}(G_1^m, G_2^f) &= \text{Cov}_{\{Y_1^m, Y_2^f\}} \left( \mathbb{E}[G_1^m | \{Y_1^m, Y_2^f\}], \mathbb{E}[G_2^f | \{Y_1^m, Y_2^f\}] \right) \\ 1654 \quad &+ \mathbb{E}_{\{Y_1^m, Y_2^f\}} \left[ \text{Cov}(G_1^m, G_2^f | \{Y_1^m, Y_2^f\}) \right] \\ 1655 \quad &= \text{Cov}_{\{Y_1^m, Y_2^f\}} \left( \mathbb{E}[G_1^m | Y_1^m], \mathbb{E}[G_2^f | Y_2^f] \right) \quad [\text{from Eqs. A.25 and A.26}] \\ 1656 \quad &= \text{Cov}(h_1^2 Y_1^m, h_2^2 Y_2^f) \quad [\text{from Eq. (A.27)}] \\ 1657 \quad &= h_1^2 h_2^2 \text{Cov}(Y_1^m, Y_2^f). \end{aligned} \quad (\text{A.28})$$

1659 Similarly,  $\text{Cov}(G_2^m, G_1^f) = h_1^2 h_2^2 \text{Cov}(Y_2^m, Y_1^f)$ .

1660 Let  $V^1$  and  $V^2$  be the phenotypic variances of traits 1 and 2, and  $V_G^1$  and  $V_G^2$  their additive genetic  
1661 variances, assumed to be the same across the sexes. Given the calculations above, the correlation among  
1662 mates for their breeding values of traits 1 and 2,  $\rho_G$ , can be written

$$1663 \quad \rho_G = \frac{\frac{1}{2} [\text{Cov}(G_1^m, G_2^f) + \text{Cov}(G_2^m, G_1^f)]}{\sqrt{V_G^1 V_G^2}} \quad (\text{A.29})$$

$$1664 \quad \begin{aligned} &= \frac{\frac{h_1^2 h_2^2}{2} [\text{Cov}(Y_1^m, Y_2^f) + \text{Cov}(Y_2^m, Y_1^f)]}{\sqrt{h_1^2 V^1 h_2^2 V^2}} \\ 1665 \quad &= h_1 h_2 \frac{\frac{1}{2} [\text{Cov}(Y_1^m, Y_2^f) + \text{Cov}(Y_2^m, Y_1^f)]}{\sqrt{V^1 V^2}} = h_1 h_2 \rho. \end{aligned} \quad (\text{A.30})$$

1667 When traits 1 and 2 are the same, we have  $\rho_G = h^2 \rho$ , a standard result (e.g., Wright 1921; Felsenstein  
1668 1981).

1669 Expanding the numerator of Eq. (A.29),

$$1670 \quad \begin{aligned} \frac{1}{2} [\text{Cov}(G_1^m, G_2^f) + \text{Cov}(G_2^m, G_1^f)] &= \frac{1}{2} [\text{Cov}(G_1^{m,\text{mat}} + G_1^{m,\text{pat}}, G_2^{f,\text{mat}} + G_2^{f,\text{pat}}) \\ 1671 \quad &+ \text{Cov}(G_2^{m,\text{mat}} + G_2^{m,\text{pat}}, G_1^{f,\text{mat}} + G_1^{f,\text{pat}})] \\ 1672 \quad &= \frac{1}{2} [\text{Cov}(G_1^{m,\text{mat}}, G_2^{f,\text{mat}}) + \text{Cov}(G_1^{m,\text{mat}}, G_2^{f,\text{pat}})] + \frac{1}{2} [\text{Cov}(G_1^{m,\text{pat}}, G_2^{f,\text{mat}}) + \text{Cov}(G_1^{m,\text{pat}}, G_2^{f,\text{pat}})] \\ 1673 \quad &+ \frac{1}{2} [\text{Cov}(G_2^{m,\text{mat}}, G_1^{f,\text{mat}}) + \text{Cov}(G_2^{m,\text{mat}}, G_1^{f,\text{pat}})] + \frac{1}{2} [\text{Cov}(G_2^{m,\text{pat}}, G_1^{f,\text{mat}}) + \text{Cov}(G_2^{m,\text{pat}}, G_1^{f,\text{pat}})] \\ 1674 \quad &= \frac{1}{2} [\text{Cov}(G_1^{m,\text{mat}}, G_2^{f,\text{mat}}) + \text{Cov}(G_2^{m,\text{mat}}, G_1^{f,\text{mat}})] + \frac{1}{2} [\text{Cov}(G_1^{m,\text{mat}}, G_2^{f,\text{pat}}) + \text{Cov}(G_2^{m,\text{mat}}, G_1^{f,\text{pat}})] \\ 1675 \quad &+ \frac{1}{2} [\text{Cov}(G_1^{m,\text{pat}}, G_2^{f,\text{mat}}) + \text{Cov}(G_2^{m,\text{pat}}, G_1^{f,\text{mat}})] + \frac{1}{2} [\text{Cov}(G_1^{m,\text{pat}}, G_2^{f,\text{pat}}) + \text{Cov}(G_2^{m,\text{pat}}, G_1^{f,\text{pat}})]. \end{aligned} \quad (\text{A.31})$$

1676

1677 But

$$\begin{aligned}
 1678 \quad & \frac{1}{2} \left[ \text{Cov} \left( G_1^{\text{m,mat}}, G_2^{\text{f,mat}} \right) + \text{Cov} \left( G_2^{\text{m,mat}}, G_1^{\text{f,mat}} \right) \right] = \frac{1}{2} \left[ \text{Cov} \left( \sum_{l \in L} g_l^{\text{m,mat}} \alpha_l, \sum_{l' \in L} g_{l'}^{\text{f,mat}} \beta_{l'} \right) \right. \\
 1679 \quad & \left. + \text{Cov} \left( \sum_{l \in L} g_l^{\text{m,mat}} \beta_l, \sum_{l' \in L} g_{l'}^{\text{f,mat}} \alpha_{l'} \right) \right] \\
 1680 \quad & = \frac{1}{2} \left[ \sum_{l \in L} \sum_{l' \in L} \text{Cov} \left( g_l^{\text{m,mat}}, g_{l'}^{\text{f,mat}} \right) \alpha_l \beta_{l'} + \sum_{l \in L} \sum_{l' \in L} \text{Cov} \left( g_l^{\text{m,mat}}, g_{l'}^{\text{f,mat}} \right) \alpha_{l'} \beta_l \right] \\
 1681 \quad & = \frac{1}{2} \left[ \sum_{l \in L} \sum_{l' \in L} \text{Cov} \left( g_l^{\text{m,mat}}, g_{l'}^{\text{f,mat}} \right) \alpha_l \beta_{l'} + \sum_{l \in L} \sum_{l' \in L} \text{Cov} \left( g_{l'}^{\text{m,mat}}, g_l^{\text{f,mat}} \right) \alpha_l \beta_{l'} \right] \\
 1682 \quad & = \sum_{l \in L} \sum_{l' \in L} \frac{1}{2} \left[ \text{Cov} \left( g_l^{\text{m,mat}}, g_{l'}^{\text{f,mat}} \right) + \text{Cov} \left( g_{l'}^{\text{m,mat}}, g_l^{\text{f,mat}} \right) \right] \alpha_l \beta_{l'} \\
 1683 \quad & = \sum_{l \in L} \sum_{l' \in L} \tilde{D}_{ll'} \alpha_l \beta_{l'}, \\
 1684 \quad &
 \end{aligned}$$

1685 since grandmaternal and grandpaternal alleles are transmitted to the offspring with equal probability,  
 1686 independently across maternal and paternal transmission. The three additional terms in Eq. (A.31)  
 1687 likewise each amount to  $\sum_{l \in L} \sum_{l' \in L} \tilde{D}_{ll'} \alpha_l \beta_{l'}$ , and so

$$1688 \quad \frac{1}{2} \left[ \text{Cov} \left( G_1^{\text{m}}, G_2^{\text{f}} \right) + \text{Cov} \left( G_2^{\text{m}}, G_1^{\text{f}} \right) \right] = 4 \sum_{l \in L} \sum_{l' \in L} \tilde{D}_{ll'} \alpha_l \beta_{l'}. \quad (\text{A.32})$$

1689 Noting that the trans-covariance at a given locus  $\tilde{D}_{ll} = p_l(1-p_l)\tilde{r}_{ll}$ , where  $\tilde{r}_{ll}$  is the within-locus correlation  
 1690 (equal to the inbreeding coefficient at the locus), we can split Eq. (A.32) into within- and between-locus  
 1691 terms:

$$1692 \quad \frac{1}{2} \left[ \text{Cov} \left( G_1^{\text{m}}, G_2^{\text{f}} \right) + \text{Cov} \left( G_2^{\text{m}}, G_1^{\text{f}} \right) \right] = 4 \sum_{l \in L} p_l(1-p_l)\tilde{r}_{ll}\alpha_l\beta_l + 4 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} \tilde{D}_{ll'} \alpha_l \beta_{l'}. \quad (\text{A.33})$$

1693 In the denominator of Eq. (A.29),

$$1694 \quad V_G^1 = \text{Var} \left( G_1^{\text{m}} \right) = \text{Var} \left( G_1^{\text{m,mat}} + G_1^{\text{m,pat}} \right) = \text{Var} \left( G_1^{\text{m,mat}} \right) + \text{Var} \left( G_1^{\text{m,pat}} \right) + 2\text{Cov} \left( G_1^{\text{m,mat}}, G_1^{\text{m,pat}} \right), \quad (\text{A.34})$$

1695 Expanding the first term,

$$\begin{aligned}
 1696 \quad \text{Var} \left( G_1^{\text{m,mat}} \right) & = \text{Var} \left( \sum_{l \in L} g_l^{\text{m,mat}} \alpha_l \right) = \sum_{l \in L} \text{Var} \left( g_l^{\text{m,mat}} \right) \alpha_l^2 + \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} \text{Cov} \left( g_l^{\text{m,mat}}, g_{l'}^{\text{m,mat}} \right) \alpha_l \alpha_{l'} \\
 1697 \quad & = \sum_{l \in L} p_l(1-p_l)\alpha_l^2 + \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D'_{ll'} \alpha_l \alpha_{l'}. \\
 1698 \quad &
 \end{aligned}$$

1699 Similarly, the second term is

$$1700 \quad \text{Var} \left( G_1^{\text{m,pat}} \right) = \sum_{l \in L} p_l(1-p_l)\alpha_l^2 + \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D'_{ll'} \alpha_l \alpha_{l'}.$$

1701 The third, covariance term in Eq. (A.34) is

$$\begin{aligned}
 1702 \quad \text{Cov} \left( G_1^{\text{m,mat}}, G_1^{\text{m,pat}} \right) &= \text{Cov} \left( \sum_{l \in L} g_l^{\text{m,mat}} \alpha_l, \sum_{l' \in L} g_{l'}^{\text{m,pat}} \alpha_{l'} \right) = \sum_{l \in L} \sum_{l' \in L} \text{Cov} \left( g_l^{\text{m,mat}}, g_{l'}^{\text{m,pat}} \right) \alpha_l \alpha_{l'} \\
 1703 \quad &= \sum_{l \in L} \sum_{l' \in L} \frac{1}{2} \left[ \text{Cov} \left( g_l^{\text{m,mat}}, g_{l'}^{\text{m,pat}} \right) + \text{Cov} \left( g_{l'}^{\text{m,mat}}, g_l^{\text{m,pat}} \right) \right] \alpha_l \alpha_{l'} \\
 1704 \quad &= \sum_{l \in L} \sum_{l' \in L} \tilde{D}'_{ll'} \alpha_l \alpha_{l'} = \sum_{l \in L} p_l (1 - p_l) \tilde{r}'_{ll} \alpha_l^2 + \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} \tilde{D}'_{ll'} \alpha_l \alpha_{l'}.
 \end{aligned}$$

1706 Putting these together in Eq. (A.34),

$$1707 \quad V_G^1 = \text{Var} (G_1^{\text{m}}) = 2 \sum_{l \in L} p_l (1 - p_l) (1 + \tilde{r}'_{ll}) \alpha_l^2 + 2 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} (D'_{ll'} + \tilde{D}'_{ll'}) \alpha_l \alpha_{l'}.$$

1708 Similarly,

$$1709 \quad V_G^2 = \text{Var} (G_2^{\text{m}}) = 2 \sum_{l \in L} p_l (1 - p_l) (1 + \tilde{r}'_{ll}) \beta_l^2 + 2 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} (D'_{ll'} + \tilde{D}'_{ll'}) \beta_l \beta_{l'}.$$

1710 In equilibrium,  $D'_{ll'} = \tilde{D}'_{ll'} = \tilde{D}_{ll'} = D_{ll'}^*$  for  $l \neq l'$ , and  $\tilde{r}'_{ll} = \tilde{r}_{ll} = \tilde{r}_{ll}^*$ , so

$$1711 \quad \frac{1}{2} \left[ \text{Cov} \left( G_1^{\text{m}}, G_2^{\text{f}} \right) + \text{Cov} \left( G_2^{\text{m}}, G_1^{\text{f}} \right) \right] = 4 \sum_{l \in L} p_l (1 - p_l) r_{ll}^* \alpha_l \beta_l + 4 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D_{ll'}^* \alpha_l \beta_{l'}, \quad (\text{A.35})$$

$$1712 \quad V_G^1 = 2 \sum_{l \in L} p_l (1 - p_l) (1 + \tilde{r}_{ll}^*) \alpha_l^2 + 4 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D_{ll'}^* \alpha_l \alpha_{l'} + V_E^1 \approx V_g^1 + 4 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D_{ll'}^* \alpha_l \alpha_{l'}, \quad (\text{A.36})$$

$$1713 \quad V_G^2 = 2 \sum_{l \in L} p_l (1 - p_l) (1 + \tilde{r}_{ll}^*) \beta_l^2 + 4 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D_{ll'}^* \beta_l \beta_{l'} + V_E^2 \approx V_g^2 + 4 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D_{ll'}^* \beta_l \beta_{l'}, \quad (\text{A.37})$$

1714  
 1715 where  $V_g^1$  and  $V_g^2$  are the genic variances of traits 1 and 2, and the approximations come from the fact  
 1716 that, under assortative mating for a polygenic trait, the sum of the  $\sim |L|^2$  cross-locus trans-LD terms  $\tilde{D}_{ll'}^*$   
 1717 dominates the sum of the  $|L|$  within-locus trans-LD terms  $\tilde{D}_{ll}^* = p_l (1 - p_l) \tilde{r}_{ll}^*$  (Crow and Kimura 1970,  
 1718 Ch. 4). Eq. (A.29) in equilibrium is therefore

$$\begin{aligned}
 1719 \quad \rho_G &= \frac{4 \sum_{l \in L} p_l (1 - p_l) \tilde{r}_{ll}^* \alpha_l \beta_l + 4 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D_{ll'}^* \alpha_l \beta_{l'}}{\sqrt{V_G^1 V_G^2}} \\
 1720 \quad &\approx \frac{4 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D_{ll'}^* \alpha_l \beta_{l'}}{\sqrt{\left( V_g^1 + 4 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D_{ll'}^* \alpha_l \alpha_{l'} \right) \left( V_g^2 + 4 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D_{ll'}^* \beta_l \beta_{l'} \right)}}. \quad (\text{A.38})
 \end{aligned}$$

1722 We now consider some special cases.

1723 **Same-trait assortative mating with equal effect sizes.** In the case of same-trait assortative mating,  
1724  $\alpha_l = \beta_l$ , so Eq. (A.38) simplifies to

$$1725 \quad \rho_G = \frac{4 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D_{ll'}^* \alpha_l \alpha_{l'}}{V_g + 4 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D_{ll'}^* \alpha_l \alpha_{l'}}, \quad (\text{A.39})$$

1726 from which

$$1727 \quad 4 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D_{ll'}^* \alpha_l \alpha_{l'} \approx \frac{\rho_G}{1 - \rho_G} V_g \quad \left( = \frac{h^2 \rho}{1 - h^2 \rho} V_g \right). \quad (\text{A.40})$$

1728 Since, in equilibrium,  $D_{ll'} = \tilde{D}_{ll'}$ , this expression can also be written

$$1729 \quad 2 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} \left( D_{ll'}^* + \tilde{D}_{ll'}^* \right) \alpha_l \alpha_{l'} \approx \frac{\rho_G}{1 - \rho_G} V_g. \quad (\text{A.41})$$

1730 Because the additive genetic variance  $V_G = V_g + 2 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} \left( D_{ll'}^* + \tilde{D}_{ll'}^* \right) \alpha_l \alpha_{l'}$ , Eq. (A.41) can also be  
1731 written

$$1732 \quad V_G = V_g / (1 - \rho_G), \quad (\text{A.42})$$

1733 which is a classic result (e.g., Wright 1921; Crow and Kimura 1970, Ch. 4).

1734 If we make the further assumption that effect sizes are the same across loci ( $\alpha_l = \alpha$  for all  $l \in L$ ),  
1735 then Eq. (A.41) becomes

$$1736 \quad 2 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} \left( D_{ll'}^* + \tilde{D}_{ll'}^* \right) \approx \frac{1}{\alpha^2} \frac{\rho_G}{1 - \rho_G} V_g. \quad (\text{A.43})$$

1737 In a population association study at locus  $l$ , assuming no indirect effects and no sources of genetic  
1738 confounding other than assortative mating, the effect size estimate is

$$1739 \quad \hat{\alpha}_l = \alpha_l + \frac{2}{V_l} \sum_{\substack{l' \in L \\ l' \neq l}} \left( D_{ll'}^* + \tilde{D}_{ll'}^* \right) \alpha_{l'},$$

1740 so that the proportionate bias in the effect size estimate at  $l$  is

$$1741 \quad \frac{\hat{\alpha}_l - \alpha_l}{\alpha_l} = \frac{2}{V_l} \sum_{\substack{l' \in L \\ l' \neq l}} \left( D_{ll'}^* + \tilde{D}_{ll'}^* \right) \frac{\alpha_{l'}}{\alpha_l} = \frac{2}{H_l} \sum_{\substack{l' \in L \\ l' \neq l}} \left( D_{ll'}^* + \tilde{D}_{ll'}^* \right), \quad (\text{A.44})$$

1742 since  $\alpha_{l'} = \alpha_l$  by assumption and  $V_l \approx H_l = 2p_l(1 - p_l)$  because assortative mating does not substantially  
1743 increase within-locus homozygosity (Crow and Kimura 1970, Ch. 4). The average proportionate bias

1744 across loci is then

$$\begin{aligned}
 1745 \quad \frac{1}{|L|} \sum_{l \in L} \frac{\hat{\alpha}_l - \alpha_l}{\alpha_l} &= \frac{1}{|L|} \sum_{l \in L} \frac{2}{\bar{H}_l} \sum_{\substack{l' \in L \\ l' \neq l}} (D_{ll'}^* + \tilde{D}_{ll'}^*) \\
 1746 &\approx \frac{2}{|L|\bar{H}} \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} (D_{ll'}^* + \tilde{D}_{ll'}^*) \\
 1747 &\approx \frac{1}{|L|\bar{H}\alpha^2} \frac{\rho_G}{1 - \rho_G} V_g \\
 1748 &= \frac{1}{V_g} \frac{\rho_G}{1 - \rho_G} V_g \\
 1749 &= \frac{\rho_G}{1 - \rho_G}, \tag{A.45} \\
 1750
 \end{aligned}$$

1751 where we have used Eq. (A.43) and have assumed that minor allele frequencies do not differ widely  
 1752 across loci. Since  $\rho_G = h^2\rho$ , where  $\rho$  is the phenotypic correlation among mates and  $h^2 = V_G/V_P$  is the  
 1753 heritability of the trait, Eq. (A.45) can also be written

$$1754 \quad \frac{1}{|L|} \sum_{l \in L} \frac{\hat{\alpha}_l - \alpha_l}{\alpha_l} = \frac{h^2\rho}{1 - h^2\rho}. \tag{A.46}$$

1755 **Sex-symmetric cross-trait assortative mating with distinct genetic bases and equal effect**  
 1756 **sizes.** In the case of cross-trait assortative mating, if the sets of loci underlying the two traits,  $L_1$  and  
 1757  $L_2$ , are distinct, then  $\alpha_l \neq 0 \Rightarrow \beta_l = 0$  and  $\beta_l \neq 0 \Rightarrow \alpha_l = 0$ . In this case, Eq. (A.38) becomes

$$1758 \quad \rho_G = \frac{4 \sum_{l \in L_1} \sum_{l' \in L_2} D_{ll'}^* \alpha_l \beta_{l'}}{\sqrt{V_G^1 V_G^2}}, \tag{A.47}$$

1759 from which

$$1760 \quad \rho_G \sqrt{V_G^1 V_G^2} = 4 \sum_{l \in L_1} \sum_{l' \in L_2} D_{ll'}^* \alpha_l \beta_{l'} = 2 \sum_{l \in L_1} \sum_{l' \in L_2} (D_{ll'}^* + \tilde{D}_{ll'}^*) \alpha_l \beta_{l'}. \tag{A.48}$$

1761 Because assortative mating is cross-trait, the LDs that assortative mating induces across  $L_1$  and  $L_2$   
 1762 will dominate the second-order LDs induced within  $L_1$  and within  $L_2$ . Therefore,  $V_G^1 \approx V_g^1$  and  $V_G^2 \approx V_g^2$ .

1763 The effect size estimate at a locus  $l \in L_1$  in a population GWAS on trait 2 is

$$1764 \quad \hat{\beta}_l \approx \frac{2}{\bar{V}_l} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}) \beta_{l'} \approx \frac{2}{\bar{H}_l} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}) \beta_{l'}, \tag{A.49}$$

1765 while the true effect size  $\beta_l$  is zero, since  $l \notin L_2$ . In equilibrium, the average effect size estimate, and thus  
 1766 the average deviation of these estimates from the true values, is therefore

$$1767 \quad \frac{1}{|L_1|} \sum_{l \in L_1} \hat{\beta}_l \approx \frac{1}{|L_1|} \sum_{l \in L_1} \frac{2}{\bar{H}_l} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}) \beta_{l'} \approx \frac{2}{|L_1|\bar{H}_1} \sum_{l \in L_1} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}) \beta_{l'}, \tag{A.50}$$



1768 where we have assumed that minor allele frequencies are not very different across  $L_1$  ( $\bar{H}_1$  is the average  
1769 heterozygosity in  $L_1$ ). If we further assume that effect sizes at causal loci are equal for each trait ( $\alpha_l = \alpha$   
1770 for all  $l \in L_1$  and  $\beta_{l'} = \beta$  for all  $l' \in L_2$ ), then Eq. (A.50) can be written

$$\begin{aligned}
 1771 \quad \frac{1}{|L_1|} \sum_{l \in L_1} \hat{\beta}_l &\approx \frac{2}{|L_1| \bar{H}_1} \sum_{l \in L_1} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}) \beta \\
 1772 &= \frac{\alpha}{|L_1| \bar{H}_1 \alpha^2} \times 2 \sum_{l \in L_1} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}) \alpha \beta \\
 1773 &= \frac{\alpha}{V_g^1} \times \rho_G \sqrt{V_G^1 V_G^2} \quad [\text{from Eq. A.48}] \\
 1774 &\approx \rho_G \sqrt{\frac{V_G^1}{V_G^2}} \alpha = \sqrt{\frac{V_G^1}{V_P^1} \cdot \frac{V_G^2}{V_P^2}} \rho \sqrt{\frac{V_G^1}{V_G^2}} \alpha = \rho \frac{V_G^1}{\sqrt{V_P^1 V_P^2}} \alpha, \tag{A.51} \\
 1775
 \end{aligned}$$

1776 recalling from Eq. (A.30) that  $\rho_G = \sqrt{h_1 h_2} \rho$ .

1777 In the further special case where both the genetic and the phenotypic variances of the two traits are  
1778 equal, then so are the heritabilities of the two traits. In this case, Eq. (A.51) simplifies to

$$1779 \quad \frac{1}{|L_1|} \sum_{l \in L_1} \hat{\beta}_l \approx \frac{V_G}{V_P} \rho \alpha = h^2 \rho \alpha, \tag{A.52}$$

1780 where  $h^2$  is the common heritability of the two traits.

### 1781 Sex-symmetric cross-trait assortative mating for traits with different genetic architectures.

1782 Eq. (A.52) reveals an interesting role for genetic architecture in the bias that cross-trait assortative mating  
1783 can generate in population association studies performed at non-causal loci. Suppose, as we did in deriving  
1784 Eq. (A.52), that the two traits on which assortative mating is based have the same genetic and phenotypic  
1785 variances,  $V_G$  and  $V$ , and therefore also the same heritabilities,  $h^2$ . We shall make the further assumption  
1786 that the traits have the same genic variance,  $V_g$ . Assume further that the sets of loci underlying traits 1  
1787 and 2,  $L_1$  and  $L_2$ , have similar mean heterozygosities  $\approx \bar{H}$ . Normalize the effect size sizes at loci causal  
1788 for trait 2 to  $\beta = 1$ , so that the traits' common genic variance is  $V_g = |L_2| \bar{H}$ .

1789 Suppose that we now perform a population GWAS for trait 2. At loci that are causal for trait 2  
1790 ( $l \in L_2$ ), we will estimate effect sizes accurately:  $\hat{\beta}_l \approx 1$  (there will be a small positive second-order bias,  
1791 of order  $\rho^2$ , since the locus  $l \in L_2$  comes into positive LD with loci  $l' \in L_1$ , which in turn have come into  
1792 positive LD with loci  $l'' \in L_2$ ).

1793 At loci that are causal for trait 1 ( $l \in L_1$ ), and which therefore have no effect on trait 2, we will  
1794 estimate effect sizes on average as given by Eq. (A.52):  $\hat{\beta}_l = h^2 \rho \alpha$ .

1795 How does the number of loci underlying variation in trait 1,  $|L_1|$ , affect this biased estimate of their  
1796 effect on trait 2? For the genic variance of trait 1 to be the same as that of trait 2,  $V_g = |L_1| \bar{H} \alpha^2 =$   
1797  $|L_2| \bar{H} \beta^2 = |L_2| \bar{H}$ , and so we must have  $\alpha^2 = |L_2|/|L_1|$ . Substituting this into the average effect size  
1798 estimate at non-causal loci,  $\hat{\beta}_l = h^2 \rho \sqrt{|L_2|/|L_1|}$ .

1799 So, the average effect size estimate at causal loci  $l \in L_2$  is  $\hat{\beta}_l \approx 1$ , while the average effect size estimate  
1800 at non-causal loci  $l \in L_1$  is  $\hat{\beta}_l = h^2 \rho \sqrt{|L_2|/|L_1|}$ . How do these two quantities compare? If the number of  
1801 loci underlying the two traits is the same,  $L_1 = L_2$ , and effect size estimates at non-causal loci are smaller  
1802 than those at causal loci by a factor of about  $h^2 \rho$ . However, if there are more loci underlying trait 2 than

1803 underlying trait 1—i.e., if trait 1 has a more concentrated genetic architecture  $L_1 < L_2$ —then the effect  
 1804 size estimates at non-causal loci will be closer to those at causal loci. Indeed, if trait 1 has a sufficiently  
 1805 concentrated architecture relative to trait 2, specifically, if  $L_1 < h^4 \rho^2 L_2$ , then the effect size estimates at  
 1806 non-causal loci will, on average, be larger in magnitude than effect size estimates at causal loci.

1807 More generally, the calculations above suggest that, in a more realistic scenario where effect sizes vary  
 1808 across loci, the trait-2 GWAS distribution of magnitudes of effect size estimates at trait-1 loci (non-causal)  
 1809 will overlap more with the distribution of magnitudes of effect size estimates at trait-2 loci (causal) if the  
 1810 genetic architecture of trait 1 is more concentrated (Fig. 4). This will lead to a greater number of trait 1  
 1811 loci being identified as statistically significantly associated with trait 2 in the trait-2 GWAS.

### 1812 A3.1.2 Cross-trait assortative mating that is asymmetric with respect to sex

1813 We now consider the case where the strength of assortative mating between two traits, as measured by  
 1814 their correlation coefficient across mating pairs, is not equal in the female-male and male-female directions.  
 1815 This is clearest in the case of an active mate preference exhibited by one sex for some phenotype exhibited  
 1816 by the other sex.

1817 To study this case, we make several simplifying assumptions. First, we assume that the genetic bases  
 1818 of variation in the two traits are distinct:  $\alpha_l \neq 0 \Leftrightarrow \beta_l = 0$ . Second we assume that there is only one  
 1819 active direction of assortative mating: female trait 1 and male trait 2. That is, conditional on the mother's  
 1820 breeding value for trait 1 and the father's breeding value for trait 2, there is no correlation between the  
 1821 mother's breeding value for trait 2 and the father's breeding value for trait 1:

$$1822 \quad \text{Cov} \left( G_2^m, G_1^f \mid \{G_1^m, G_2^f\} \right) = 0.$$

1823 Suppose that there is a constant correlation  $\rho_G$  between mothers' breeding values for trait 1 and  
 1824 fathers' breeding values for trait 2:

$$1825 \quad \rho_G = \frac{\text{Cov} \left( G_1^m, G_2^f \right)}{\sqrt{V_G^1 V_G^2}}. \quad (\text{A.53})$$

1826 To study the genetic consequences of this assortment, we need to know the average bi-directional corre-  
 1827 lation among mates for traits 1 and 2 (Eq. A.29). Since traits 1 and 2 will come into a positive genetic  
 1828 correlation via assortative mating of female trait 1 and male trait 2, there will also be a positive covariance  
 1829 between mothers' breeding values for trait 2 and fathers' breeding values for trait 1, which we can express  
 1830 using the law of total covariance:

$$1831 \quad \begin{aligned} \text{Cov} \left( G_2^m, G_1^f \right) &= \text{Cov}_{\{G_1^m, G_2^f\}} \left( \mathbb{E} \left[ G_2^m \mid \{G_1^m, G_2^f\} \right], \mathbb{E} \left[ G_1^f \mid \{G_1^m, G_2^f\} \right] \right) \\ &+ \mathbb{E}_{\{G_1^m, G_2^f\}} \left[ \text{Cov} \left( G_2^m, G_1^f \mid \{G_1^m, G_2^f\} \right) \right] \\ &= \text{Cov}_{\{G_1^m, G_2^f\}} \left( \mathbb{E} \left[ G_2^m \mid G_1^m \right], \mathbb{E} \left[ G_1^f \mid G_2^f \right] \right). \end{aligned} \quad (\text{A.54})$$

1835 If  $G_1^m$  and  $G_2^m$  are bivariate normal (more generally, if  $G_2^m = a + bG_1^m + \varepsilon$ , with  $\mathbb{E}[\varepsilon] = \mathbb{E}[\varepsilon G_1^m] = 0$ ), then

$$1836 \quad \begin{aligned} \mathbb{E} \left[ G_2^m \mid G_1^m \right] &= \mathbb{E} \left[ G_2^m \right] + \rho_{m1,m2} \sqrt{V_G^2/V_G^1} \left( G_1^m - \mathbb{E} \left[ G_1^m \right] \right) \\ &= \mathbb{E} \left[ G_2^m \right] + \rho_{m1,m2} \left( G_1^m - \mathbb{E} \left[ G_1^m \right] \right), \end{aligned}$$

1839 where  $\rho_{m1,m2} = \text{Corr}(G_1^m, G_2^m)$  is the genetic correlation between traits 1 and 2 in mothers, and where we  
 1840 have assumed that the two traits have equal variance. Similarly, if  $G_1^f$  and  $G_2^f$  are bivariate normal, then

$$1841 \quad \mathbb{E} \left[ G_1^f \mid G_2^f \right] = \mathbb{E} \left[ G_1^f \right] + \rho_{f1,f2} \left( G_2^f - \mathbb{E} \left[ G_2^f \right] \right).$$

1842 Substituting these expressions into Eq. (A.54),

$$1843 \quad \text{Cov} \left( G_2^m, G_1^f \right) = \rho_{m1,m2} \rho_{f1,f2} \text{Cov} \left( G_1^m, G_2^f \right). \quad (\text{A.55})$$

1844 But, in our case,  $\rho_{m1,m2} = \rho_{f1,f2}$ , the common value of which we shall call  $\rho_{12}$ , and so the average  
 1845 bi-directional correlation is

$$1846 \quad \frac{\frac{1}{2} [\text{Cov} (G_1^m, G_2^f) + \text{Cov} (G_2^m, G_1^f)]}{\sqrt{V_G^1 V_G^2}} = \frac{\frac{1}{2} (1 + \rho_{12}^2) \text{Cov} (G_1^m, G_2^f)}{\sqrt{V_G^1 V_G^2}} = \frac{\rho_G}{2} (1 + \rho_{12}^2). \quad (\text{A.56})$$

1847 Given this value, the calculations of the effect of assortative mating on the weighted sums of cis- and trans-  
 1848 covariances, and thus on the additive genetic variance, proceed as for the case of symmetric assortative  
 1849 mating above.

1850 Assuming the genetic bases of the two traits to be distinct, we may substitute the average bi-directional  
 1851 correlation,  $\rho_G (1 + \rho_{12}^2) / 2$ , into Eq. (A.48) to find

$$1852 \quad \rho_G (1 + \rho_{12}^2) = \frac{4 \sum_{l \in L_1} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}) \alpha_l \beta_{l'}}{\sqrt{V_G^1 V_G^2}}. \quad (\text{A.57})$$

1853 But

$$1854 \quad \rho_{12} = \frac{2 \sum_{l \in L_1} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}) \alpha_l \beta_{l'}}{\sqrt{V_G^1 V_G^2}},$$

1855 and so Eq. (A.57) can be written as the quadratic equation  $\rho_G (1 + \rho_{12}^2) = 2\rho_{12}$ , the relevant solution  
 1856 to which is  $\rho_{12} = \left( 1 - \sqrt{1 - \rho_G^2} \right) / \rho_G$ . If  $\rho_G$  is small, we use the first-order Taylor approximation  
 1857  $\sqrt{1 - \rho_G^2} \approx 1 - \rho_G^2 / 2$  to find

$$1858 \quad \frac{\rho_G}{2} \approx \rho_{12} = \frac{2 \sum_{l \in L_1} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}) \alpha_l \beta_{l'}}{\sqrt{V_G^1 V_G^2}} \approx \frac{2 \sum_{l \in L_1} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}) \alpha_l \beta_{l'}}{\sqrt{V_g^1 V_g^2}}. \quad (\text{A.58})$$

1859 In the particular scenario we have simulated in Fig. 2,  $V_g^1 = V_g^2$ ,  $\alpha_l = 1$  for all  $l \in L_1$ , and  $\beta_l = 1$  for  
 1860 all  $l \in L_2$ , so Eq. (A.58) further simplifies to

$$1861 \quad 4 \sum_{l \in L_1} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}) = \rho V_g^1 \quad (\text{A.59})$$

1862 In a population association study for trait 2 performed at a locus  $l \in L_1$  (so that  $\beta_l = 0$ ),

$$1863 \quad \hat{\beta}_l = \beta_l + \frac{2}{V_l} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}) \beta_{l'} = \frac{2}{V_l} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}). \quad (\text{A.60})$$

1864 Across loci in  $L_1$ , the average estimate is

$$1865 \quad \bar{\hat{\beta}}_l = \frac{1}{|L_1|} \sum_{l \in L_1} \frac{2}{V_l} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}). \quad (\text{A.61})$$

1866 In our simulations,  $p_l \approx 1/2$  for all  $l$  so that  $V_l \approx 2p_l(1 - p_l) = 1/2$ , and  $|L_1| = |L_2| = 500$ , so  
 1867  $V_g^1 = V_g^2 = 250$ . Under this configuration,

$$1868 \quad \begin{aligned} \bar{\hat{\beta}}_l &= \frac{1}{|L_1|} \sum_{l \in L_1} \frac{2}{V_l} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}) = \frac{1}{500} \sum_{l \in L_1} \frac{2}{1/2} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}) \\ 1869 &= \frac{4}{500} \sum_{l \in L_1} \sum_{l' \in L_2} (D_{ll'} + \tilde{D}_{ll'}) = \frac{\rho_G V_g^1}{500} = \rho_G/2. \end{aligned}$$

1870

1871 The trait we simulated is genetic, with heritability 1, and so  $\rho_G = \rho$ , the phenotypic correlation among  
 1872 mates. We chose a strength of assortative mating of  $\rho = 0.2$ , and so, in equilibrium, the average effect  
 1873 size estimate at non-causal loci should be approximately 0.1, which is indeed the case in Fig. 2.

### 1874 **Sex-asymmetric cross-trait assortative mating for traits with different genetic architectures.**

1875 For the case where the numbers of loci underlying traits 1 and 2 differ, and noting that the ‘effective’  
 1876 correlation among mates in the sex-asymmetric case is approximately half that in the sex-symmetric case  
 1877 (Eq. A.58), we can perform a similar back-of-the-envelope calculation as in the sex-symmetric cross-trait  
 1878 assortative mating case above to find that, when effect sizes are constant across trait-1 loci and constant  
 1879 across trait-2 loci (though differing across traits 1 and 2), the effect size estimates at trait-1 (non-causal)  
 1880 loci in a trait-2 population GWAS is, on average, a fraction  $\frac{h^2 \rho}{2} \sqrt{|L_2|/|L_1|}$  of the estimates at trait-2  
 1881 (causal) loci.

1882 Thus, more generally, when the number of loci underlying trait 1 is small relative to the number of  
 1883 loci underlying trait 2, the distribution of magnitudes of effect size estimates at trait-1 loci in a trait-2  
 1884 GWAS can overlap substantially with the distribution of magnitudes of effect size estimates at trait-2 loci  
 1885 (Fig. 4), causing variants at these non-causal trait-1 loci to show up as significant in the trait-2 GWAS.

### 1886 **A3.2 Population structure**

1887 In the model we have considered, with results displayed in Fig. 5, there are initially two isolated pop-  
 1888 ulations of equal size. The frequency of the focal variant at locus  $l$  is  $p_l^{(1)}$  in population 1 and  $p_l^{(2)}$  in  
 1889 population 2, so that its overall frequency is  $p_l = (p_l^{(1)} + p_l^{(2)})/2$ . A population GWAS at locus  $\lambda$  returns  
 1890 an effect size estimate

$$1891 \quad \hat{\alpha}_\lambda^{\text{pop}} = \frac{2}{V_\lambda} \sum_{l \in L} (D_{\lambda l} + \tilde{D}_{\lambda l}) \alpha_l,$$

1892 where  $D_{\lambda l}$  and  $\tilde{D}_{\lambda l}$  are calculated across both populations and are generally nonzero because of allele  
 1893 frequency differences between the two populations at loci  $\lambda$  and  $l$  (Nei and Li 1973). In our case,

$$1894 \quad V_\lambda = 2p_\lambda(1 - p_\lambda)(1 + F_\lambda),$$

1895 and

$$1896 \quad D_{\lambda l} = \tilde{D}_{\lambda l} = \frac{1}{4} (p_\lambda^{(1)} - p_\lambda^{(2)}) (p_l^{(1)} - p_l^{(2)}),$$

1897 SO

$$1898 \quad \hat{\alpha}_\lambda^{\text{pop}} = \frac{p_\lambda^{(1)} - p_\lambda^{(2)}}{2p_\lambda(1-p_\lambda)(1+F_\lambda)} \sum_{l \in L} (p_l^{(1)} - p_l^{(2)}) \alpha_l.$$

1899 Squaring this and multiplying by  $2p_\lambda(1-p_\lambda)$ ,

$$2p_\lambda(1-p_\lambda)(\hat{\alpha}_\lambda^{\text{pop}})^2 = \frac{(p_\lambda^{(1)} - p_\lambda^{(2)})^2}{2p_\lambda(1-p_\lambda)(1+F_\lambda)^2} \left[ \sum_{l \in L} (p_l^{(1)} - p_l^{(2)})^2 \alpha_l^2 + \sum_{l \neq l'} (p_l^{(1)} - p_l^{(2)}) (p_{l'}^{(1)} - p_{l'}^{(2)}) \alpha_l \alpha_{l'} \right].$$

1900 (A.62)

1901 **Neutral allele frequency divergence.** If allele frequency divergence between the two populations is  
 1902 neutral, frequency changes at different loci are independent of one another and of effect sizes, so the second  
 1903 term in square brackets above is zero in expectation. In addition, because Hardy-Weinberg equilibrium  
 1904 obtains within each population, non-zero expected values of  $F_\lambda$  derive only from allele frequency differences  
 1905 between the populations, so that  $F_\lambda = F_{ST,\lambda}$  in expectation. Therefore,

$$1906 \quad \mathbb{E} [2p_\lambda(1-p_\lambda)(\hat{\alpha}_\lambda^{\text{pop}})^2] = \frac{1}{(1+F_{ST})^2} \mathbb{E} \left[ \frac{(p_\lambda^{(1)} - p_\lambda^{(2)})^2}{2p_\lambda(1-p_\lambda)} \mid L \mid \mathbb{E} \left[ (p_l^{(1)} - p_l^{(2)})^2 \right] \mathbb{E} [\alpha_l^2] \right]$$

$$1907 \quad = \frac{1}{(1+F_{ST})^2} \mathbb{E} [2F_{ST,\lambda} \mid L \mid \mathbb{E} [2F_{ST,l} H_l] \mathbb{E} [\alpha_l^2]]$$

$$1908 \quad \approx \frac{4|L|}{(1+F_{ST})^2} (\mathbb{E} [F_{ST,l}]^2 \mathbb{E} [H_l] \mathbb{E} [\alpha_l^2])$$

$$1909 \quad = 4|L| \left( \frac{F_{ST}}{1+F_{ST}} \right)^2 \mathbb{E} [H_l] \mathbb{E} [\alpha_l^2],$$

1910

1911 where  $H_l = 2p_l(1-p_l)$ . If the ancestral allele frequency at  $l$  was  $p_l^a$ , then  $\mathbb{E}[H_l | p_l^a] = 2p_l^a(1-p_l^a)(1-F_{ST,l})$ ,  
 1912 and so  $\mathbb{E}[H_l]$  is calculated using the law of iterated expectations by averaging this quantity over the  
 1913 ancestral distribution of allele frequencies:  $\mathbb{E}[H_l] \approx \mathbb{E}[H_l^a](1-F_{ST})$ , where  $H_l^a = 2p_l^a(1-p_l^a)$ . So

$$1914 \quad \mathbb{E} [2p_\lambda(1-p_\lambda)(\hat{\alpha}_\lambda^{\text{pop}})^2] \approx 4|L| \left( \frac{F_{ST}}{1+F_{ST}} \right)^2 (1-F_{ST}) \mathbb{E} [H_l^a] \mathbb{E} [\alpha_l^2]. \quad (\text{A.63})$$

1915 **Selection and phenotype-biased migration.** Above, in calculating the mean heterozygosity-weighted  
 1916 value of  $(\hat{\alpha}_\lambda)^2$  under neutral frequency divergence between populations, we assumed that in Eq. (A.62)  
 1917 the second term in the square brackets was zero, i.e., that the effect-size-signed population allele fre-  
 1918 quency difference was uncorrelated across loci. However, when selection or phenotype-biased migra-  
 1919 tion acts, this will no longer be true. For example, if higher genetic values of the trait were favoured  
 1920 in population 1 relative to population 2, then selection will on average have driven a mean shift such  
 1921 that  $\mathbb{E} \left[ (p_l^{(1)} - p_l^{(2)}) \alpha_l \right] > 0$ . This in turn will drive systematic positive covariances between terms  
 1922  $(p_l^{(1)} - p_l^{(2)}) \alpha_l$  and  $(p_{l'}^{(1)} - p_{l'}^{(2)}) \alpha_{l'}$ , and as these covariances are summed over all pairs of loci in  
 1923 Eq. (A.62), the resulting inflation of the average squared effect size estimate (and other genome-wide  
 1924 summaries) could be quantitatively substantial.

1925 **More general population stratification.** Given a sample of  $N$  individuals, the sample cis-LD be-  
 1926 tween two markers  $\lambda$  and  $l$  can be written generally as

$$1927 \quad D_{\lambda l} = \frac{1}{N-1} \sum_{i=1}^N \left( \Delta g_{i,\lambda}^m \Delta g_{i,l}^m + \Delta g_{i,\lambda}^p \Delta g_{i,l}^p \right), \quad (\text{A.64})$$

1928 where  $\Delta g_{i,k}^m$  and  $\Delta g_{i,k}^p$  are the deviations of individual  $i$ 's maternal and paternal focal allele count at locus  
 1929  $k$  from their mean frequencies. The trans-LD between  $\lambda$  and  $l$  is

$$1930 \quad \tilde{D}_{\lambda l} = \frac{1}{N-1} \sum_{i=1}^N \left( \Delta g_{i,\lambda}^m \Delta g_{i,l}^p + \Delta g_{i,\lambda}^p \Delta g_{i,l}^m \right). \quad (\text{A.65})$$

1931 These cis- and trans-LD terms are equal only if

$$1932 \quad D_{\lambda l} - \tilde{D}_{\lambda l} = \frac{1}{N-1} \sum_{i=1}^N \left( \Delta g_{i,\lambda}^m - \Delta g_{i,\lambda}^p \right) \left( \Delta g_{i,l}^m - \Delta g_{i,l}^p \right) = 0, \quad (\text{A.66})$$

1933 i.e., if the maternal and paternal alleles at the one locus are exchangeable with respect to deviations of  
 1934 the allelic state at the other locus.

1935 We might often be concerned with stratification along some specific axis of variation in our sample. Call  
 1936 this axis  $v$ , with every individual having a value along  $v$ , with mean zero across individuals (for example,  
 1937 in our two population case above, the vector  $v$  could be 1 for population 1 and  $-1$  for population 2). The  
 1938 covariance of the maternal allele at locus  $l$  with the vector  $v$  is proportional to  $a_l^m \cdot v = \sum_i a_{i,l}^m v_i$ . So the  
 1939 contribution of LD along this axis to the difference in cis- and trans-LD is

$$1940 \quad D_{\lambda l}^{(v)} - \tilde{D}_{\lambda l}^{(v)} = \left( (\Delta g_{\lambda}^m - \Delta g_{\lambda}^p) \cdot v \right) \left( (\Delta g_l^m - \Delta g_l^p) \cdot v \right), \quad (\text{A.67})$$

1941 which is zero only if the maternal and paternal genotypes at the two loci are exchangeable with respect  
 1942 to each other along the axis  $v$ .

### 1943 A3.3 Admixture

1944 Suppose that two previously isolated populations admix in proportions  $A$  and  $1 - A$ , with subsequent  
 1945 random mating in the admixed population. Following the notation in the Section A3.2 above, before  
 1946 admixture, the frequency of the focal variant at locus  $l$  was  $p_l^{(1)}$  in population 1 and  $p_l^{(2)}$  in population 2,  
 1947 so that its overall frequency in the admixed population is  $p_l = A p_l^{(1)} + (1 - A) p_l^{(2)}$ .

1948 When the two populations admix, trans-LD between all pairs of loci disappears in expectation, owing  
 1949 to random mating in the admixed population:  $\tilde{D}_{\lambda l}^t = 0$  for any pairs of loci  $\lambda$  and  $l$  and for any number  
 1950 of generations  $t$  after admixture. However, cis-associations between alleles that were more prevalent in  
 1951 one ancestral population than in the other will be retained as cis-LD in the admixed population until  
 1952 these associations are eroded by recombination. The initial degree of cis-LD between loci  $\lambda$  and  $l$  in the  
 1953 admixed population is

$$1954 \quad D_{\lambda l}^0 = A(1 - A) \left( p_{\lambda}^{(1)} - p_{\lambda}^{(2)} \right) \left( p_l^{(1)} - p_l^{(2)} \right).$$

1955 When  $t$  generations have elapsed since admixture, this cis-LD will have been eroded by recombination to

$$1956 \quad D_{\lambda l}^t = D_{\lambda l}^0 (1 - c_{\lambda l})^t = A(1 - A) \left( p_{\lambda}^{(1)} - p_{\lambda}^{(2)} \right) \left( p_l^{(1)} - p_l^{(2)} \right) (1 - c_{\lambda l})^t,$$

1957 where  $c_{\lambda l}$  is the sex-averaged recombination rate between  $\lambda$  and  $l$ . Therefore,  $t$  generations after admixture,  
1958 a population association study at  $\lambda$  returns an effect size estimate

$$1959 \quad \hat{\alpha}_{\lambda}^{\text{pop},t} = \frac{2}{V_{\lambda}} \sum_{l \in L} D_{\lambda l}^t \alpha_l = A(1-A) \frac{p_{\lambda}^{(1)} - p_{\lambda}^{(2)}}{p_{\lambda}(1-p_{\lambda})} \sum_{l \in L} \left( p_l^{(1)} - p_l^{(2)} \right) (1-c_{\lambda l})^t \alpha_l,$$

1960 while a sibling-based association study at  $\lambda$  returns

$$1961 \quad \hat{\alpha}_{\lambda}^{\text{sib},t} = \frac{2}{H_{\lambda}} \sum_{l \in L} (1-2c_{\lambda l}) D_{\lambda l}^t \alpha_l = A(1-A) \frac{p_{\lambda}^{(1)} - p_{\lambda}^{(2)}}{p_{\lambda}(1-p_{\lambda})} \sum_{l \in L} \left( p_l^{(1)} - p_l^{(2)} \right) (1-c_{\lambda l})^t (1-2c_{\lambda l}) \alpha_l,$$

1962 where we have substituted  $V_{\lambda} = H_{\lambda} = 2p_{\lambda}(1-p_{\lambda})$  owing to random mating in the admixed population.

1963 Squaring the population estimate and multiplying by  $2p_{\lambda}(1-p_{\lambda})$ ,

$$1964 \quad 2p_{\lambda}(1-p_{\lambda})(\hat{\alpha}_{\lambda}^{\text{pop},t})^2 = 2A^2(1-A)^2 \frac{\left( p_{\lambda}^{(1)} - p_{\lambda}^{(2)} \right)^2}{p_{\lambda}(1-p_{\lambda})} \left[ \sum_{l \in L} \left( p_l^{(1)} - p_l^{(2)} \right)^2 (1-c_{\lambda l})^{2t} \alpha_l^2 \right. \\ 1965 \quad \left. + \sum_{l \neq l'} \left( p_l^{(1)} - p_l^{(2)} \right) \left( p_{l'}^{(1)} - p_{l'}^{(2)} \right) (1-c_{\lambda l})^t (1-c_{\lambda l'})^t \alpha_l \alpha_{l'} \right], \quad (\text{A.68})$$

1967 while the heterozygosity-weighted squared sibling effect size is

$$1968 \quad 2p_{\lambda}(1-p_{\lambda})(\hat{\alpha}_{\lambda}^{\text{sib},t})^2 = 2A^2(1-A)^2 \frac{\left( p_{\lambda}^{(1)} - p_{\lambda}^{(2)} \right)^2}{p_{\lambda}(1-p_{\lambda})} \left[ \sum_{l \in L} \left( p_l^{(1)} - p_l^{(2)} \right)^2 (1-c_{\lambda l})^{2t} (1-2c_{\lambda l})^2 \alpha_l^2 \right. \\ 1969 \quad \left. + \sum_{l \neq l'} \left( p_l^{(1)} - p_l^{(2)} \right) \left( p_{l'}^{(1)} - p_{l'}^{(2)} \right) (1-c_{\lambda l})^t (1-c_{\lambda l'})^t (1-2c_{\lambda l})(1-2c_{\lambda l'}) \alpha_l \alpha_{l'} \right]. \quad (\text{A.69})$$

1971 **Neutral allele frequency divergence.** If allele frequency divergence between the two populations  
1972 was neutral, then frequency changes at different loci are independent of one another, of effect sizes, and of  
1973 recombination rates (assuming the loci are sufficiently far apart), so the second terms in square brackets  
1974 in Eqs. (A.68) above is zero in expectation, so that

$$1975 \quad \mathbb{E} \left[ 2p_{\lambda}(1-p_{\lambda})(\hat{\alpha}_{\lambda}^{\text{pop},t})^2 \right] = 4A^2(1-A)^2 \mathbb{E} \left[ \frac{\left( p_{\lambda}^{(1)} - p_{\lambda}^{(2)} \right)^2}{2p_{\lambda}(1-p_{\lambda})} \mid L \right] \mathbb{E} \left[ \left( p_l^{(1)} - p_l^{(2)} \right)^2 \right] \overline{(1-c)^{2t}} \mathbb{E} \left[ \alpha_l^2 \right] \\ 1976 \quad = 4A^2(1-A)^2 \overline{(1-c)^{2t}} \mathbb{E} [2F_{ST,\lambda} \mid L] \mathbb{E} [2F_{ST,l} H_l] \mathbb{E} [\alpha_l^2] \\ 1977 \quad \approx 16A^2(1-A)^2 \overline{(1-c)^{2t}} |L| F_{ST}^2 \mathbb{E} [H_l] \mathbb{E} [\alpha_l^2],$$

1979 where  $\overline{(1-c)^{2t}}$  is the average value of  $(1-c_{ll'})^{2t}$  taken across all pairs of loci  $l, l'$ .

1980 Similarly, under drift in the ancestral populations, the average squared sibling-based effect size estimate  
1981 can be simplified to

$$1982 \quad \mathbb{E} \left[ 2p_{\lambda}(1-p_{\lambda})(\hat{\alpha}_{\lambda}^{\text{sib},t})^2 \right] \approx 16A^2(1-A)^2 \overline{(1-c)^{2t} (1-2c)^2} |L| F_{ST}^2 \mathbb{E} [H_l] \mathbb{E} [\alpha_l^2],$$

1983 where  $\overline{(1-c)^{2t} (1-2c)^2}$  is the average value of  $(1-c_{ll'})^{2t} (1-2c_{ll'})$  taken across all pairs of loci  $l, l'$ .

1984 **Selection and phenotype-biased migration.** As in the case of population structure, selection and  
 1985 phenotype-biased migration in the ancestral populations can drive systematic positive covariances between  
 1986 the terms  $(p_l^{(1)} - p_l^{(2)}) \alpha_l$  and  $(p_{l'}^{(1)} - p_{l'}^{(2)}) \alpha_{l'}$  in Eqs. (A.68) and (A.69) above, so that the second terms  
 1987 in square brackets in these equations do not cancel in expectation as they did under neutral divergence  
 1988 between the ancestral populations. Again, as these covariances are summed over all pairs of loci in  
 1989 Eqs. (A.68) and (A.69), the resulting inflation of the average squared effect size estimate and other  
 1990 genome-wide summaries could be substantial.

### 1991 A3.4 Stabilizing selection

1992 We consider the model of Bulmer (1971, 1974), in which a very large number of loci contribute variation  
 1993 to a trait under stabilizing selection. We assume that the distribution of trait values is centered on the  
 1994 optimal value  $Y^*$ , and that the relative fitness of an individual with trait value  $Y$  is  $\exp(-(Y - Y^*)^2/2V_S)$ ,  
 1995 where  $V_S$ , the width or ‘variance’ of this gaussian selection function, governs the strength of stabilizing  
 1996 selection, with larger  $V_S$  values implying weaker selection. Under this model, selection acts to reduce the  
 1997 phenotypic variation each generation; if the trait value is normally distributed with variance  $V_P$ , then  
 1998 selection reduces the within-generation phenotypic variance by an amount

$$1999 \Delta V_P = \frac{-V_P^2}{V_S + V_P}. \quad (\text{A.70})$$

2000 How much of this reduction carries over to the offspring generation then depends on the heritability of  
 2001 the trait.

2002 Owing to the large number of loci in this model, the buildup of LD among them occurs on a faster  
 2003 timescale than the change in allele frequencies at individual loci. Assuming the loci to have equal effect  
 2004 sizes, Bulmer (1974) showed that the overall reduction in the phenotypic variance due to stabilizing  
 2005 selection,  $d$ , rapidly approaches a quasi-equilibrium value that approximately satisfies

$$2006 d^* = \frac{1}{2} h^{*4} \Delta V_P^* / \bar{c}_h, \quad (\text{A.71})$$

2007 where  $h^{*2}$  is the heritability of the trait in this equilibrium and  $\bar{c}_h$  is the harmonic mean of the recom-  
 2008 bination rates amongst all pairs of loci. On this rapid timescale, the reduction in variance is due to LD  
 2009 among the loci underlying the trait; in fact,

$$2010 d = 2\alpha^2 \sum_{l \in L} \sum_{l' \in L} D_{ll'}, \quad (\text{A.72})$$

2011 where  $\alpha$  is the common per-locus effect size and  $D_{ll'}$  is defined with respect to the trait-increasing alleles  
 2012 at  $l$  and  $l'$ . The individual linkage disequilibria  $D_{ll'}$ , in expectation, are proportional to the inverse  
 2013 recombination rates  $1/c_{ll'}$ . Writing

$$2014 2\alpha^2 \sum_{l \in L} \sum_{l' \in L} D_{ll'}^* = d^* = \frac{1}{2} h^{*4} \Delta V_P^* / \bar{c}_h = \frac{1}{2} h^{*4} \Delta V_P^* \frac{\sum_l \sum_{l' \neq l} 1/c_{ll'}}{\binom{L}{2}}, \quad (\text{A.73})$$

2015 where  $\binom{L}{2} = |L|(|L| - 1)/2$  is the number of pairs of distinct loci in  $L$ , it is apparent that

$$2016 \mathbb{E}[D_{ll'}^*] = \frac{1}{4\alpha^2} h^{*4} \Delta V_P^* \frac{1/c_{ll'}}{\binom{L}{2}}. \quad (\text{A.74})$$



Henceforth we deal only with equilibrium quantities and therefore drop the star superscript for neatness. The phenotypic variance  $V_P$  can be written  $V_P = V_G + V_E = V_g + d + V_E$ , where  $V_G$  is the additive genetic variance,  $V_g$  is the genic variance, and  $V_E$  is the variance due to the environment. Eqs. (A.70) and (A.71), together with the definition of heritability  $h^2 = V_G/V_P$ , define a quadratic equation in  $d$ :

$$(1 + 2H)d^2 + 2[(V_S + V_g + V_E)\bar{c}_h + V_g]d + V_g^2 = 0. \quad (\text{A.75})$$

Eq. (A.75) matches Eq. (10) in Bulmer (1974), with Bulmer's parameter  $c$  replaced by  $1/2V_S$ . For ease of reference in what follows, we write Eq. (A.75) in the standard form  $ad^2 + bd + c = 0$ . The roots are

$$d_{+,-} = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} = \frac{-[(V_S + V_g + V_E)\bar{c}_h + V_g] \pm \sqrt{[(V_S + V_g + V_E)\bar{c}_h + V_g]^2 - (1 + 2H)V_g^2}}{1 + 2\bar{c}_h}. \quad (\text{A.76})$$

To see which of these roots is the relevant one, we first note that the roots are both real, since the requirement for this is

$$\begin{aligned} [(V_S + V_g + V_E)\bar{c}_h + V_g]^2 \geq (1 + 2\bar{c}_h)V_g^2 &\Leftrightarrow (V_S + V_g + V_E)\bar{c}_h + V_g \geq \sqrt{1 + 2\bar{c}_h}V_g \\ &\Leftrightarrow V_S + V_E \geq \frac{\sqrt{1 + 2\bar{c}_h} - 1 - \bar{c}_h}{\bar{c}_h}V_g, \end{aligned}$$

and  $\sqrt{1 + 2\bar{c}_h} < 1 + \bar{c}_h$  for  $\bar{c}_h > 0$ , while  $V_S + V_E > 0$ . Furthermore, since  $b > 0$  and  $4ac > 0$ , both roots are in fact negative, with  $d_- < d_+ < 0$ . Now note that

$$\begin{aligned} 2d_- < d_+ + d_- &= -\frac{b}{a} = -\frac{2[(V_S + V_g + V_E)\bar{c}_h + V_g]}{1 + 2\bar{c}_h} \\ &< -\frac{2[(V_g + V_g + V_E)\bar{c}_h + V_g]}{1 + 2\bar{c}_h} \quad (\text{since } V_g < V_S) \\ &< -\frac{2[(V_g + V_g)\bar{c}_h + V_g]}{1 + 2\bar{c}_h} \quad (\text{since } V_E > 0) \\ &= -2V_g, \end{aligned}$$

i.e.,  $V_g + d_- < 0$ . But then if the relevant root were  $d = d_-$ ,  $0 \leq V_G = V_g + d_- < 0$ , a contradiction. So the relevant root is in fact

$$d = d_+ = \frac{-[(V_S + V_g + V_E)\bar{c}_h + V_g] + \sqrt{[(V_S + V_g + V_E)\bar{c}_h + V_g]^2 - (1 + 2\bar{c}_h)V_g^2}}{1 + 2\bar{c}_h}, \quad (\text{A.77})$$

from which

$$-\frac{d}{V_g} = \frac{1 - \bar{c}_h \left( \sqrt{1 + 2 \left(1 + \frac{1}{\bar{c}_h}\right) X + X^2} - (1 + X) \right)}{1 + 2\bar{c}_h}, \quad (\text{A.78})$$

where  $X = \frac{V_S + V_E}{V_g}$ . Since, in the absence of selection,  $V_G = V_g$ , Eq. (A.78) gives the proportionate reduction in the additive genetic variance due to selection.

From Eq. (A.72),  $d = 2\alpha^2 \sum_l \sum_{l' \neq l} D_{ll'}$ , and, since  $V_g = \sum_l 2p_l(1 - p_l)\alpha^2 = \alpha^2 \bar{H}|L|$ , with  $|L|$  the number of loci and  $\bar{H}$  the average heterozygosity across them, we have

$$\frac{d}{V_g} = \frac{2 \sum_l \sum_{l' \neq l} D_{ll'}}{\bar{H}L}. \quad (\text{A.79})$$

2047 In a population association study performed at locus  $l$ , the effect size estimate is

$$2048 \hat{\alpha}_l^{\text{pop}} = \alpha_l + \frac{2}{2p_l(1-p_l)} \sum_{l' \neq l} D_{ll'} \alpha_{l'} = \alpha \left( 1 + \frac{2}{2p_l(1-p_l)} \sum_{l' \neq l} D_{ll'} \right), \quad (\text{A.80})$$

2049 so that the proportionate error is

$$2050 \frac{2}{2p_l(1-p_l)} \sum_{l' \neq l} D_{ll'}. \quad (\text{A.81})$$

2051 The mean proportionate error across loci is therefore

$$2052 \frac{1}{|L|} \sum_{l \in L} \left( \frac{2}{2p_l(1-p_l)} \sum_{l' \neq l} D_{ll'} \right) \approx \frac{2 \sum_l \sum_{l' \neq l} D_{ll'}}{\bar{H}|L|} = \frac{d}{V_g}, \quad (\text{A.82})$$

2053 from Eq. (A.79), and assuming that the heterozygosities do not vary much across loci. That is, the average  
2054 proportionate bias to effect size estimation that stabilizing selection induces is approximately equal to the  
2055 proportionate reduction in the additive genetic variance, which is given in general form by Eq. (A.78).

2056 In a within-family association study performed at locus  $l$ , the effect size estimate is

$$2057 \hat{\alpha}_l^{\text{fam}} = \alpha_l + \frac{2}{2p_l(1-p_l)} \sum_{l' \neq l} (1 - 2c_{ll'}) D_{ll'} \alpha_{l'} = \alpha \left( 1 + \frac{2}{2p_l(1-p_l)} \sum_{l' \neq l} (1 - 2c_{ll'}) D_{ll'} \right), \quad (\text{A.83})$$

2058 so that the proportionate error is

$$2059 \frac{2}{2p_l(1-p_l)} \sum_{l' \neq l} (1 - 2c_{ll'}) D_{ll'}. \quad (\text{A.84})$$

2060 The mean proportionate error across loci is therefore

$$\begin{aligned} 2061 \frac{1}{|L|} \sum_{l \in L} \left( \frac{2}{2p_l(1-p_l)} \sum_{l' \neq l} (1 - 2c_{ll'}) D_{ll'} \right) &\approx \frac{2 \sum_l \sum_{l' \neq l} (1 - 2c_{ll'}) D_{ll'}}{\bar{H}|L|} \\ 2062 &\approx \frac{2 \sum_l \sum_{l' \neq l} (1 - 2c_{ll'}) \frac{d\bar{c}_h}{2\alpha^2 \binom{|L|}{2} c_{ll'}}}{\bar{H}|L|} \\ 2063 &= \frac{d\bar{c}_h}{\alpha^2 \bar{H}|L| \binom{|L|}{2}} \sum_l \sum_{l' \neq l} \left( \frac{1}{c_{ll'}} - 2 \right) \\ 2064 &= \frac{d\bar{c}_h}{V_g \binom{|L|}{2}} \left( \frac{\binom{|L|}{2}}{\bar{c}_h} - 2 \binom{|L|}{2} \right) \\ 2065 &= \frac{d}{V_g} (1 - 2\bar{c}_h), \quad (\text{A.85}) \\ 2066 \end{aligned}$$

2067 where we have used Eq. (A.74) in the second line. Therefore, the mean error in the within-family GWAS  
2068 is smaller in magnitude than that in a population GWAS by a factor  $1 - 2\bar{c}_h$ .

2069 If  $\sim 1,000$  loci underlie variation in the trait (and all contribute approximately the same variation),  
2070  $\bar{c}_h \approx 0.4640$  in humans (see Methods), and so the average bias that stabilizing selection induces in within-  
2071 family GWASs will be about  $1 - 2\bar{c}_h \approx 7\%$  that in population GWASs. If  $\sim 10,000$  loci underlie variation

2072 in the trait,  $\bar{c}_h \approx 0.4346$ , and so the bias in within-family GWASs will be about 13% that in population  
2073 GWASs.

2074 The calculations above give the average proportionate bias to GWAS estimates in terms of the basic  
2075 parameters of the model,  $V_g$ ,  $V_E$ ,  $V_S$ , and  $\bar{c}_h$ . Often, however, not all of these parameters will be  
2076 measurable. For example, human height appears to be under stabilizing selection (Sanjak et al. 2018),  
2077 is highly heritable, and this heritability is believed to be underlain largely by *direct* genetic effects (Lee  
2078 et al. 2018). However, it is difficult to directly measure the genic variance in height  $V_g$  because not all  
2079 causal loci will be assayed in association studies—and, moreover, even if they were, effect size estimation  
2080 at these causal loci would be biased by the genetic confounds that we have studied in this paper. However,  
2081 the phenotypic variance in height  $V_P$  can obviously be measured, and the heritability of height  $h^2$  can  
2082 also be measured using classical methods rather than effect size estimation in association studies. The  
2083 strength of stabilizing selection on height can also be measured (Sanjak et al. 2018). From  $V_P$  and  $h^2$ ,  
2084 the additive genetic variance  $V_G$  can be estimated ( $V_G = h^2 V_P$ ).

2085 This example suggests that, in many applications, it might be useful to be able to estimate the equi-  
2086 librium value of  $d$  using  $V_G$  (or  $V_P$ ),  $V_E$ ,  $V_S$ , and  $\bar{c}_h$ , even though  $V_G$  (and  $V_P$ ), in the model we have  
2087 considered, is a state variable influenced by the state variable of primary interest,  $d$ . This is straightfor-  
2088 ward: returning to our use of a star superscript to denote equilibrium values, if we treat  $V_G$  and  $V_P$  as  
2089 their equilibrium values  $V_G^*$  and  $V_P^*$ , Eq. (A.71) can be estimated directly, and also simplifies to

$$2090 \quad d^* = -\frac{1}{2\bar{c}_h} \cdot \frac{V_G^{*2}}{V_S + V_G^* + V_E} = -\frac{1}{2\bar{c}_h} \cdot \frac{V_G^{*2}}{V_S + V_P^*} = \frac{1}{2\bar{c}_h} \cdot \frac{h^{*4} V_P^{*2}}{V_S + V_P^*}. \quad (\text{A.86})$$

2091 The proportionate bias in a population GWAS, given by Eq. (A.82), can similarly be estimated from  $h^2$ ,  
2092  $V_P$ ,  $V_S$ , and  $\bar{c}_h$ , by first observing that

$$2093 \quad V_g = V_G^* - d^* = V_G^* + \frac{1}{2\bar{c}_h} \frac{V_G^{*2}}{V_S + V_P^*} = V_G^* \left( 1 + \frac{1}{2\bar{c}_h} \cdot \frac{V_G^*}{V_S + V_P^*} \right),$$

2094 so that Eq. (A.82) can be written

$$2095 \quad \frac{d^*}{V_g} = \frac{-\frac{1}{2\bar{c}_h} \cdot \frac{V_G^{*2}}{V_S + V_P^*}}{V_G^* \left( 1 + \frac{1}{2\bar{c}_h} \cdot \frac{V_G^*}{V_S + V_P^*} \right)} = \frac{-\frac{1}{2\bar{c}_h} \cdot \frac{V_G^*}{V_S + V_P^*}}{1 + \frac{1}{2\bar{c}_h} \cdot \frac{V_G^*}{V_S + V_P^*}} = \frac{-\frac{1}{2\bar{c}_h} \cdot \frac{h^{*2} V_P^*}{V_S + V_P^*}}{1 + \frac{1}{2\bar{c}_h} \cdot \frac{h^{*2} V_P^*}{V_S + V_P^*}} = -\frac{1}{2\bar{c}_h \left( \frac{1 + V_S/V_P^*}{h^{*2}} \right) + 1}, \quad (\text{A.87})$$

2096 which reveals that the proportionate bias depends only on  $\bar{c}_h$ ,  $h^{*2}$  and the scaled inverse strength of  
2097 selection,  $V_S/V_P^*$ .

2098 From Eq. (A.85), the proportionate bias in a within-family GWAS is then approximately

$$2099 \quad \frac{d^*}{V_g} (1 - 2\bar{c}_h) = -\frac{1 - 2\bar{c}_h}{2\bar{c}_h \left( \frac{1 + V_S/V_P^*}{h^{*2}} \right) + 1}. \quad (\text{A.88})$$

## 2100 **Stabilizing selection attenuates estimates of the strength of assortative mating based on** 2101 **cross-chromosome PGS correlations**

2102 Recently, the strength of assortative mating has been estimated based on measurement of the correlation  
2103 of polygenic scores across distinct sets of chromosomes (e.g., Yengo et al. 2018; Yamamoto et al. 2023).  
2104 Were assortative mating acting in isolation, such correlations would be due entirely to the positive cis-

2105 and trans-LDs among same-effect alleles created by assortative mating. Since stabilizing selection, acting  
 2106 in isolation, generates negative cis-LDs among same-effect alleles, it will attenuate the positive cis-LDs  
 2107 generated by assortative mating, and therefore reduce the correlation in PGSs among distinct sets of  
 2108 chromosomes, leading to underestimates of the strength of assortative mating if this effect is not taken  
 2109 into account.

2110 To quantify this attenuation, we first calculate the strength of (positive) cross-chromosome LDs ex-  
 2111 pected under assortative mating alone; then we calculate the strength of (negative) cross-chromosome  
 2112 LDs expected under stabilizing selection alone; then, assuming these LDs to be generated independently  
 2113 of one another—so that the LDs generated under the joint action of assortative mating and stabilizing  
 2114 selection are the sums of the LDs expected under these forces alone—we calculate how much stabilizing  
 2115 selection attenuates the correlation in PGSs across distinct sets of chromosomes.

2116 **Cross-chromosome correlations in PGSs.** The number of autosomes in the haploid set is  $n$  ( $= 22$  in  
 2117 humans). Label the set of loci on chromosome  $k$  that contribute variation to our trait of interest  $L_k$ ; the  
 2118 overall set of loci underlying variation in the trait is  $L = \{L_1, L_2, \dots, L_k\}$ . We divide the chromosomes  
 2119 into distinct sets  $K_1$  and  $K_2$  (e.g.,  $K_1$  could be the set of odd numbered chromosomes and  $K_2$  the  
 2120 even). Let  $L^{(1)}$  and  $L^{(2)}$  be the sets of causal loci on the chromosomes in  $K_1$  and  $K_2$  respectively (i.e.,  
 2121  $L^{(i)} = \cup_{k \in K_i} L_k$ ).

2122 Suppose that we have accurately estimated effect sizes at all loci  $l \in L$ . For each individual, we then  
 2123 calculate a polygenic score for  $K_1$  and for  $K_2$ :

$$2124 \quad P_1 = \sum_{l \in L^{(1)}} g_l \alpha_l; \quad P_2 = \sum_{l' \in L^{(2)}} g_{l'} \alpha_{l'}.$$

2125 We are interested in the correlation in the population between  $P_1$  and  $P_2$ , and in particular, how this  
 2126 correlation is affected by assortative mating and stabilizing selection for the focal trait. The correlation  
 2127 can be written

$$2128 \quad \text{Corr}(P_1, P_2) = \frac{\text{Cov}(P_1, P_2)}{\sqrt{\text{Var}(P_1)\text{Var}(P_2)}},$$

2129 with

$$\begin{aligned} 2130 \quad \text{Cov}(P_1, P_2) &= \text{Cov} \left( \sum_{l \in L^{(1)}} g_l \alpha_l, \sum_{l' \in L^{(2)}} g_{l'} \alpha_{l'} \right) \\ 2131 &= \sum_{l \in L^{(1)}} \sum_{l' \in L^{(2)}} \text{Cov}(g_l, g_{l'}) \alpha_l \alpha_{l'} \\ 2132 &= 2 \sum_{l \in L^{(1)}} \sum_{l' \in L^{(2)}} \left( D_{ll'} + \tilde{D}_{ll'} \right) \alpha_l \alpha_{l'}. \end{aligned} \quad (A.89)$$

2134 Since, to make progress in the case of stabilizing selection, we will assume effect sizes to be equal across  
 2135 loci, we make that assumption now, so that

$$2136 \quad \text{Cov}(P_1, P_2) = 2\alpha^2 \sum_{l \in L^{(1)}} \sum_{l' \in L^{(2)}} \left( D_{ll'} + \tilde{D}_{ll'} \right). \quad (A.90)$$

2137 Since every pair of loci  $(l, l')$  across  $L^{(1)}$  and  $L^{(2)}$  are by definition unlinked, under many processes  
 2138 (including assortative mating and stabilizing selection), the values of  $D_{ll'}$  and  $\tilde{D}_{ll'}$  will not differ much in

2139 expectation across locus pairs, in equilibrium. Therefore, we may approximate  $D_{ll'} = D^*$  and  $\tilde{D}_{ll'} = \tilde{D}^*$   
 2140 for all  $l \in L^{(1)}$  and  $l' \in L^{(2)}$ , so that Eq. (A.90) simplifies further:

$$2141 \quad \text{Cov}(P_1, P_2) = 2 |L^{(1)}| |L^{(2)}| (D^* + \tilde{D}^*) \alpha^2. \quad (\text{A.91})$$

2142 **Assortative mating alone.** Under assortative mating with equal effect sizes across loci, in equilibrium,  
 2143 LDs are approximately equal across locus pairs, regardless of the recombination rate between them;  
 2144 moreover, cis- and trans-LDs are equal (see above). Therefore, to calculate  $D^*$  ( $= \tilde{D}^*$ ), we simply  
 2145 apportion the total LD given by Eq. (A.40) among individual locus pairs:

$$2146 \quad \frac{h^2 \rho}{1 - h^2 \rho} V_g \approx 4 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D_{ll'}^* \alpha_l \alpha_{l'} = 4 |L| (|L| - 1) \alpha^2 D^* \\
 2147 \Rightarrow D^* \approx \frac{\frac{h^2 \rho}{1 - h^2 \rho} V_g}{4 |L| (|L| - 1) \alpha^2} = \frac{\frac{h^2 \rho}{1 - h^2 \rho} |L| \bar{H} \alpha^2}{4 |L| (|L| - 1) \alpha^2} = \frac{\frac{h^2 \rho}{1 - h^2 \rho} \bar{H}}{4 (|L| - 1)} \approx \frac{1}{4} \cdot \frac{h^2 \rho}{1 - h^2 \rho} \cdot \frac{\bar{H}}{|L|}, \quad (\text{A.92})$$

2149 when  $|L|$  is large. Similarly,

$$2150 \quad \tilde{D}^* \approx \frac{1}{4} \cdot \frac{h^2 \rho}{1 - h^2 \rho} \cdot \frac{\bar{H}}{|L|}, \quad (\text{A.93})$$

2151 so that the overall contribution of assortative mating to the covariance in Eq. (A.91) is proportional to

$$2152 \quad D^* + \tilde{D}^* \approx \frac{1}{2} \cdot \frac{h^2 \rho}{1 - h^2 \rho} \cdot \frac{\bar{H}}{|L|}. \quad (\text{A.94})$$

2153 **Stabilizing selection alone.** Under stabilizing selection, the total amount of negative cis-LD is given  
 2154 by Eq. (A.87):

$$2155 \quad 2\alpha^2 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D_{ll'} = d = -\frac{V_g}{2\bar{c}_h \left( \frac{1+V_S/V_P}{h^2} \right) + 1}, \quad (\text{A.95})$$

2156 where we have dropped the equilibrium ‘\*’ markers. This expression does not easily decompose into  
 2157 terms from individual locus pairs. However, if we assume that stabilizing selection is relatively weak  
 2158 ( $V_S/V_P^* \gg 1$ ) and that the recombination process is such that the harmonic mean recombination rate  
 2159  $\bar{c}_h \sim 1/2$  (as is the case in humans), Eq. (A.95) can be approximated by

$$2160 \quad 2\alpha^2 \sum_{l \in L} \sum_{\substack{l' \in L \\ l' \neq l}} D_{ll'} = d \approx -\frac{V_g}{2\bar{c}_h \left( \frac{1+V_S/V_P}{h^2} \right)} = -\frac{1}{2} \cdot \frac{h^2 V_g}{1 + V_S/V_P} \cdot \frac{1}{\bar{c}_h} = -\frac{1}{2} \cdot \frac{h^2 V_g}{1 + V_S/V_P} \cdot \frac{2 \sum_{l,l'} 1/c_{ll'}}{|L| (|L| - 1)},$$

2161 from which we infer that, in expectation,

$$2162 \quad 2\alpha^2 D_{ll'} \approx -\frac{h^2 V_g}{1 + V_S/V_P} \cdot \frac{1/c_{ll'}}{|L| (|L| - 1)}.$$

2163 Therefore, for unlinked  $l$  and  $l'$  ( $c_{ll'} = 1/2$ ), in expectation,

$$2164 \quad D_{ll'} \approx -\frac{1}{\alpha^2 |L| (|L| - 1)} \cdot \frac{h^2 V_g}{1 + V_S/V_P} = -\frac{\bar{H}}{\alpha^2 \bar{H} |L| (|L| - 1)} \cdot \frac{h^2 V_g}{1 + V_S/V_P} = -\frac{\bar{H}}{(|L| - 1) V_g} \cdot \frac{h^2 V_g}{1 + V_S/V_P} \\
 2165 = -\frac{\bar{H}}{|L| - 1} \cdot \frac{h^2}{1 + V_S/V_P} \approx -\frac{\bar{H}}{|L|} \cdot \frac{h^2}{1 + V_S/V_P}. \quad (\text{A.96})$$

2167 Stabilizing selection does not systematically generate trans-LD, so, in expectation,  $\tilde{D}_{ll'} = 0$ . Therefore,  
 2168 under stabilizing selection alone, the contribution of an unlinked locus pair to the covariance in Eq. (A.91)  
 2169 is

$$2170 \quad D^* + \tilde{D}^* = D^* \approx -\frac{\bar{H}}{|L|} \cdot \frac{h^2}{1 + V_S/V_P}. \quad (\text{A.97})$$

2171 **How much does stabilizing selection attenuate the signal of assortative mating?** Comparing  
 2172 Eqs. (A.94) and (A.97), we find that the proportionate attenuation of assortative mating's effect (in  
 2173 isolation) by the action of stabilizing selection is

$$2174 \quad -\frac{\bar{H}}{|L|} \cdot \frac{h^2}{1 + V_S/V_P} = \frac{-2}{1 + V_S/V_P} \cdot \frac{1 - h^2\rho}{\rho}. \quad (\text{A.98})$$

2175 For example, in the case of human height ( $h^2 \sim 0.8$ ), the signal of assortative mating (strength  $\rho \sim 0.25$ )  
 2176 is attenuated by stabilizing selection (strength  $V_S/V_P \sim 30$ ) by a proportionate amount of approximately  
 2177 20%. That is, one might measure by other means (e.g., the phenotypic correlation among mates, together  
 2178 with an estimate of the heritability of height) that the strength of assortative mating is  $\rho = 0.25$ , but  
 2179 estimating this strength from cross-chromosome PGS correlations without accounting or correcting for  
 2180 stabilizing selection on height would yield  $\hat{\rho} \approx 0.2$ , 20% smaller than the true value.

## 2181 A4 One-locus GxE

2182 We study the phenotypic model in Eq. (22), with the phenotype of individual  $i$  in family  $f$  given by

$$2183 \quad Y_i = Y^* + (\alpha + \alpha_f + \alpha_i) g_i + \epsilon_f + \epsilon_i, \quad (\text{A.99})$$

2184 where, across the population,  $\mathbb{E}[\alpha_f] = \mathbb{E}[\alpha_i] = \mathbb{E}[\epsilon_f] = \mathbb{E}[\epsilon_i] = 0$ , and  $\alpha_i$ ,  $\epsilon_f$ , and  $\epsilon_i$  are all independent of  
 2185  $g_i$ .

2186 **Sibling GWAS.** Let  $i$  and  $j$  be siblings in family  $f$ , and define  $\Delta Y_f = Y_i - Y_j$ ,  $\Delta g_f = g_i - g_j$ , and  
 2187  $\Delta \epsilon_f = \epsilon_i - \epsilon_j$ . A sibling association study returns an effect size estimate

$$2188 \quad \hat{\alpha}^{\text{sib}} = \frac{\text{Cov}(\Delta Y_f, \Delta g_f)}{\text{Var}(\Delta g_f)} = \frac{\text{Cov}((\alpha + \alpha_f) \Delta g_f + (\alpha_i g_i - \alpha_j g_j) + \Delta \epsilon_f, \Delta g_f)}{\text{Var}(\Delta g_f)}$$

$$2189 \quad = \frac{\mathbb{E}[(\alpha + \alpha_f) (\Delta g_f)^2] + \mathbb{E}[(\alpha_i g_i - \alpha_j g_j) \Delta g_f] + \mathbb{E}[\Delta \epsilon_f \Delta g_f]}{H},$$

2191 where  $H$  is the fraction of parents who are heterozygous at the focal locus. Since  $\alpha_i$ ,  $\alpha_j$ ,  $\epsilon_i$ , and  $\epsilon_j$  are  
 2192 genotype-independent perturbations,  $\mathbb{E}[(\alpha_i g_i - \alpha_j g_j) \Delta g_f] = \mathbb{E}[\Delta \epsilon_f \Delta g_f] = 0$ , and so

$$2193 \quad \hat{\alpha} = \frac{\mathbb{E}[\alpha (\Delta g_f)^2] + \mathbb{E}[\alpha_f (\Delta g_f)^2]}{H} = \alpha + \frac{\mathbb{E}[\alpha_f (\Delta g_f)^2]}{H}, \quad (\text{A.100})$$

2194 which deviates from  $\alpha$  by an amount  $\mathbb{E}[\alpha_f (\Delta g_f)^2] / H$ .

2195 Let  $\Delta g_f^{\text{mat}}$  and  $\Delta g_f^{\text{pat}}$  be the difference in the genotypes of the siblings in family  $f$  due to maternal  
 2196 and paternal transmission. Because of the independence of maternal and paternal transmission in a given

2197 family, the term additional to  $\alpha$  in Eq. (A.100) can be split into  $\mathbb{E}[\alpha_f(\Delta g_f^{\text{mat}})^2]/H$  and  $\mathbb{E}[\alpha_f(\Delta g_f^{\text{pat}})^2]/H$ ,  
 2198 which we can analyze separately.

2199 If the mother is heterozygous, then  $(\Delta g_f^{\text{mat}})^2$  equals 1 with probability 1/2 and 0 with probability 1/2;  
 2200 if the mother is homozygous, then  $(\Delta g_f^{\text{mat}})^2$  is 0. Therefore, denoting by  $h^m$  the event that the mother is  
 2201 heterozygous,

$$2202 \quad \frac{\mathbb{E}[\alpha_f(\Delta g_f^{\text{mat}})^2]}{H} = \frac{\frac{1}{2}\mathbb{E}[\alpha_f | h^m] \text{Prob}(h^m)}{H} = \frac{1}{2}\mathbb{E}[\alpha_f | h^m].$$

2203 The same holds for paternal transmission, and so the deviation of the family-based estimate  $\hat{\alpha}$  from  $\alpha$  is

$$2204 \quad \hat{\alpha} - \alpha = \frac{\mathbb{E}[\alpha_f(\Delta g_f)^2]}{H} = \mathbb{E}[\alpha_f | h]. \quad (\text{A.101})$$

2205 That is, quite intuitively, if the average G×E effect  $\alpha_f$  is different in the families of heterozygous parents  
 2206 than in the population as a whole, then limiting estimation to the offspring of heterozygous parents will  
 2207 be problematic.

2208 **Population GWAS.** Under the same one-locus model, a population association study returns an effect  
 2209 size estimate of

$$2210 \quad \hat{\alpha}^{\text{pop}} = \frac{\text{Cov}(Y_i, g_i)}{\text{Var}(g_i)} = \frac{\text{Cov}((\alpha + \alpha_f + \alpha_i)g_i + \epsilon_f + \epsilon_i, g_i)}{\text{Var}(g_i)}$$

$$2211 \quad = \alpha + \frac{\text{Cov}(\alpha_f g_i, g_i)}{\text{Var}(g_i)}. \quad (\text{A.102})$$

2212 We can immediately see from Eq. (A.102) that if the family environments are randomized across genotypes,  
 2213 such that  $\alpha_f$  and  $g_i$  are independent (implying  $\text{Cov}(\alpha_f g_i, g_i) = 0$ ), then the population estimate will  
 2214 coincide with  $\alpha$ .

2215 To calculate the deviation of the population estimate from  $\alpha$  in the general case, let  $F$  be the inbreeding  
 2216 coefficient at the locus. Then  $\text{Var}(g_i) = 2p(1-p)(1+F)$ , where  $p$  is the frequency of the focal variant,  
 2217 and the frequency of heterozygotes is  $f_1 = 2p(1-p)(1-F)$  while the frequencies of the two homozygotes  
 2218 are  $f_0 = (1-p)^2 + p(1-p)F$  (zero focal alleles) and  $f_2 = p^2 + p(1-p)F$  (two focal alleles). The covariance  
 2219 term in Eq. (A.102) can then be written

$$2220 \quad \text{Cov}(\alpha_f g_i, g_i) = \mathbb{E}[\alpha_f g_i^2] - \mathbb{E}[\alpha_f g_i] \mathbb{E}[g_i] = \mathbb{E}[\alpha_f g_i^2] - 2p\mathbb{E}[\alpha_f g_i]$$

$$2221 \quad = (0 \times \mathbb{E}[\alpha_f | g_i = 0] f_0 + 1 \times \mathbb{E}[\alpha_f | g_i = 1] f_1 + 4 \times \mathbb{E}[\alpha_f | g_i = 2] f_2)$$

$$2222 \quad - 2p(0 \times \mathbb{E}[\alpha_f | g_i = 0] f_0 + 1 \times \mathbb{E}[\alpha_f | g_i = 1] f_1 + 2 \times \mathbb{E}[\alpha_f | g_i = 2] f_2)$$

$$2223 \quad = \mathbb{E}[\alpha_f | g_i = 1] f_1(1 - 2p) + 4\mathbb{E}[\alpha_f | g_i = 2] f_2(1 - p)$$

$$2224 \quad = 2\mathbb{E}[\alpha_f | g_i = 1] p(1 - p)(1 - 2p)(1 - F) + 4\mathbb{E}[\alpha_f | g_i = 2] (p^2(1 - p) + p(1 - p)^2 F).$$

2225 The deviation of the population-based estimate from  $\alpha$  is therefore

$$2226 \quad \hat{\alpha}^{\text{pop}} - \alpha = \frac{\text{Cov}(\alpha_f g_i, g_i)}{\text{Var}(g_i)}$$

$$2227 \quad = \frac{2\mathbb{E}[\alpha_f | g_i = 1] p(1 - p)(1 - 2p)(1 - F) + 4\mathbb{E}[\alpha_f | g_i = 2] (p^2(1 - p) + p(1 - p)^2 F)}{2p(1 - p)(1 + F)}$$

$$2228 \quad = \mathbb{E}[\alpha_f | g_i = 1] (1 - 2p) \frac{1 - F}{1 + F} + 2\mathbb{E}[\alpha_f | g_i = 2] (p + (1 - p)F) \frac{1}{1 + F} \quad (\text{A.103})$$

$$2229 \quad \approx \mathbb{E}[\alpha_f | g_i = 1] (1 - 2p)(1 - 2F) + 2\mathbb{E}[\alpha_f | g_i = 2] (p + (1 - 2p)F). \quad (\text{A.104})$$

2233 The approximation holds when  $F$  is small.

2234 An interesting special case is where homozygotes for the focal allele and heterozygotes have the same  
2235 distribution of environments, so that  $\mathbb{E}[\alpha_f | g_i = 1] = \mathbb{E}[\alpha_f | g_i = 2] = \mathbb{E}[\alpha_f | g_i > 0]$ . In this case,  
2236 Eq. (A.103) simplifies to

$$2237 \hat{\alpha}^{\text{POP}} - \alpha = \mathbb{E}[\alpha_f | g_i > 0], \quad (\text{A.105})$$

2238 which reveals that, if individuals who carry the focal allele tend to experience different environments to  
2239 individuals who do not carry the focal allele, then the population GWAS estimate will deviate from the  
2240 average effect under true randomization,  $\alpha$ . Moreover, in this case, if  $\mathbb{E}[\alpha_f | g_i = 1]$  and  $\mathbb{E}[\alpha_f | h]$  are the  
2241 same—that is, if the mean environment of heterozygous offspring is the same as that for heterozygous  
2242 parents—then the sibling and population-based effect size estimates are the same.