Genome-wide association study of social genetic effects on 170 phenotypes in laboratory mice

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Abstract

Social genetic effects (SGE, also called indirect genetic effects) are associations between genotypes of one individual and phenotype of another. SGE arise when two individuals interact and heritable traits of one influence the phenotype of the other. Recent studies have shown that SGE substantially contribute to phenotypic variation in humans and laboratory mice, which suggests that SGE, like direct genetic effects (DGE, effects of an individual's genes on their own phenotype), are amenable to mapping. Using 170 phenotypes including behavioural, physiological and morphological traits measured in outbred laboratory mice, we empirically explored the potential and challenges of genome-wide association study of SGE (sgeGWAS) as a tool to discover novel mechanisms of social effects between unrelated individuals. For each phenotype we performed sgeGWAS, identifying 21 genome-wide significant SGE associations for 17 phenotypes, and dgeGWAS for comparison. Our results provide three main insights: first, SGE and DGE arise from partially different loci and/or loci with different effect sizes, which implies that the widely-studied mechanism of phenotypic "contagion" is not sufficient to explain all social effects. Secondly, several DGE associations but no SGE associations had large effects, suggesting sgeGWAS

is unlikely to uncover "low hanging fruits". Finally, a similar number of variants likely contribute to SGE and DGE. The analytical framework we developed in this study and the insights we gained from our analyses will inform the design, implementation and interpretation of sgeGWAS in this and other populations and species.

Main text

Introduction

Social interactions between individuals can result in the phenotype of one individual being affected by genotypes of their social partners. Such associations arise when the phenotype of interest is influenced by heritable traits of social partners (Figure 1a), and are called social genetic effects (SGE, also called indirect genetic effects[1,2]).

SGE have been shown to contribute significantly and substantially to a range of phenotypes across species, including educational attainment in humans[3-5] and behavioural, physiological, and morphological phenotypes in laboratory mice[6-8]. One key implication of the existence of SGE is the possibility to use them as a tool to understand the mechanisms of social effects, through genome-wide association studies of SGE (sgeGWAS). Similarly to how GWAS of "traditional" direct genetic effects (DGE, effects of an individual's genotypes on its own phenotype) have provided valuable insights into the "within-body" pathways affecting disease and quantitative phenotypes[9], sgeGWAS could help dissect the "between-bodies" pathways of social effects, namely the traits of social partners that mediate social effects (Figure 1a).

To date, only a handful of studies have mapped SGE[7,8,10-12] and only three have mapped SGE genome-wide: one used inbred strains of *Drosophila melanogaster* to investigate SGE on male courtship behaviour[12] and two used inbred strains of mice to investigate SGE on maternal and pup behaviour [7,8]. Thus, sgeGWAS has never been performed in an outbred population nor for biomedical phenotypes. In order to explore the utility of sgeGWAS in this context, we analysed 170 behavioural, physiological and morphological phenotypes measured in unrelated outbred laboratory mice (Figure 1b and 1c) and performed a comparative analysis of SGE and DGE. Specifically, we quantified the correlation between SGE and DGE acting on the same phenotype in order to test whether the mechanism of phenotypic "contagion", which is the focus of

most social studies[3-5,13-17], is sufficient to explain social effects and evaluate whether sgeGWAS is likely to uncover novel mechanisms. We next performed sgeGWAS and dgeGWAS for all phenotypes in order to characterise the architecture of SGE and compare it to the architecture of DGE. Our results provide the first insights into what sgeGWAS might reveal, and will help design and interpret future sgeGWAS.

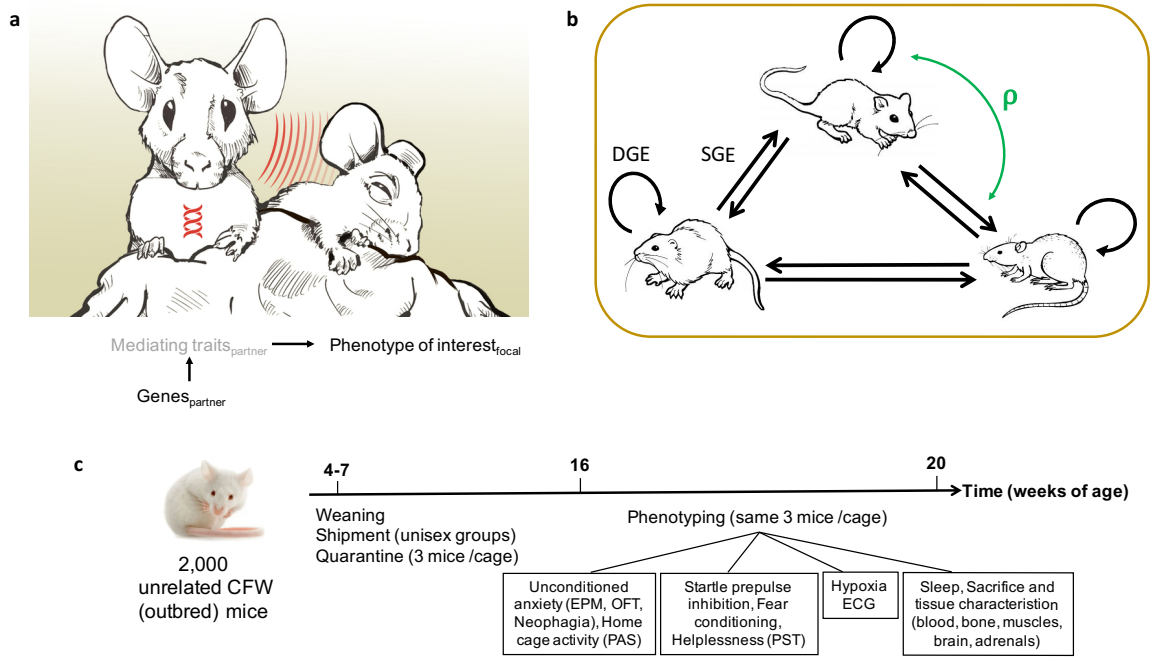

Figure 1 *Illustration of social genetic effects (SGE) and experimental design. (a) SGE arise when two individuals interact and heritable traits ("mediating traits") of one (the "social partner") influence the "phenotype of interest", measured in the "focal individual". (b) Unrelated outbred mice were housed in groups of 3 mice per cage. SGE and direct genetic effects (DGE) contributed by each mouse were modelled.* ρ *is the correlation between SGE and DGE random effects in the variance components model (see Methods). (c) Housing conditions and phenotyping.*

Results

Aggregate contribution of SGE

Because of our focus on sgeGWAS, we used data from an outbred population of mice with genetic characteristics conducive to high mapping resolution: commercially-available CFW mice[18,19]. Genome-wide genotypes and 200 behavioural, physiological and morphological phenotypes for 1,934 CFW mice were available from Nicod et al.[19] and Davies et al.[20]. We retained 1,812 mice that were unrelated and housed in cages of three mice, and 170 phenotypes that could be satisfactorily normalised (see Methods). Males were always housed with males and females with females, and mice were left undisturbed in their cages for at least nine weeks before phenotyping started (Figure 1c). The number of mice used for each phenotype, which depends on missing phenotype data, is shown in Supplementary Table 1.

As evidence of SGE in laboratory mice is still sparse[6-8], we first estimated the aggregate contribution of SGE (i.e. the sum of SGE across the genome) to each phenotype. To do so, we used the variance decomposition method detailed in Baud et al.[6], which features random effects for DGE, SGE, direct and social environmental effects, and “cage effects” (see Methods). We found significant SGE contributions for 16 out of 170 phenotypes ($P < 0.05$, FDR < 0.48), including behavioural, physiological and morphological measures (Table 1, Supplementary Table 1). SGE in aggregate explained up to 22% of variation in serum LDL levels and an average of 11% across the 16 significantly affected phenotypes. A broad range of phenotypes were affected, including measures of helplessness (a model of depression), immune status, LDL levels, platelet size, and rate of wound healing. These results show that social effects have a genetic basis in our datasets and therefore are amenable to mapping.

Name	Aggregate SGE (%)	Aggregate DGE (%)	P value SGE	Correlation ρ	P value ρ ≠ 1
PST.Immobility.First2min	13+/-4	15+/-3	0.0000099*	0.53 +/- 0.15	0.00066*
Bioch.LDL_BWcorr	22+/-6	14+/-4	0.000015*	0.84 +/- 0.1	0.025*
Adrenals.Adrenals_g_BWcorr	18+/-5	24+/-4	0.000037*	0.73 +/- 0.11	0.038*
WH.Ears_Area	17+/-4	17+/-3	0.00018*	0.65 +/- 0.12	0.0009*
Haem.MPV	14+/-5	10+/-3	0.00031*	1 +/- 0.09	1
Haem.EOS_percent	17+/-5	2+/-3	0.00065*	NA	NA
Bioch.Amylase_BWcorr	15+/-5	23+/-5	0.0013*	0.5 +/- 0.17	0.0069*
PST.Immobility.Last4min	8+/-3	18+/-3	0.0028*	0.41 +/- 0.19	0.0083*
Hypoxia.TV_AHR_BWcorr	11+/-4	8+/-4	0.0051*	-0.06 +/- 0.31	0.047*
Muscles.Gast.g_BWcorr	6+/-3	15+/-4	0.012*	0.57 +/- 0.27	0.17
Haem.MCHC	10+/-5	9+/-4	0.014*	0.66 +/- 0.25	0.16
Haem.Large_PLT	9+/-5	13+/-4	0.018*	0.88 +/- 0.15	0.39
Haem.HDW	5+/-3	24+/-4	0.023*	NA	NA
FACS.CD3posCD8posCD44pos	9+/-4	10+/-3	0.023*	0.45 +/- 0.25	0.033*
Diss.Tail.Length_BWcorr	7+/-4	15+/-4	0.043*	-0.28 +/- 0.35	0.19
Hypoxia.MV_Off_response_BWcor	4+/-4	15+/-4	0.045*	NA	NA
FACS.CD3posCD4CD8Ratio	9+/-4	32+/-5	0.059	0.34 +/- 0.18	0.03*
FACS.CD3posCD44negCD4CD8Rati	8+/-4	34+/-5	0.095	0.29 +/- 0.19	0.045*
FACS.CD3posCD8pos	6+/-4	28+/-4	0.12	0.4 +/- 0.19	0.094
FACS.CD3posCD44posCD4CD8Ratio	8+/-5	19+/-4	0.14	0.49 +/- 0.23	0.14
Bioch.Calcium_BWcorr	8+/-4	6+/-4	0.15	0.55 +/- 0.38	0.34
Hypoxia.TV_SHR_BWcorr	6+/-4	17+/-4	0.16	0.39 +/- 0.28	0.18
FACS.CD45posCD3negDX5pos	7+/-4	21+/-4	0.17	0.31 +/- 0.22	0.065
Haem.CHCM	5+/-4	8+/-4	0.22	0.71 +/- 0.33	0.43
EPM.OpenArms.Time_BWcorr	6+/-4	9+/-4	0.23	-0.14 +/- 0.36	0.13
FACS.CD45posCD3posCD4pos	7+/-4	6+/-4	0.23	0.47 +/- 0.38	0.24
Bioch.CreatinineEnzymatic_BWcor	6+/-5	8+/-4	0.34	0.65 +/- 0.34	0.36
Neuro.DCX_BWcorr	7+/-5	12+/-4	0.34	0.18 +/- 0.33	0.16
Bioch.Glucose_BWcorr	5+/-4	7+/-4	0.39	0.09 +/- 0.46	0.19
EPM.OpenArms.Distance_BWcorr	5+/-4	8+/-4	0.4	0.04 +/- 0.4	0.17
Sleep.Ampl_BWcorr	6+/-4	11+/-4	0.4	0.06 +/- 0.4	0.19
See Supplementary Table 1 for a description of the phenotypes					
* P < 0.05					

Table 1 *Variance explained by SGE and DGE in aggregate, and correlation ρ between random SGE and DGE (see Methods). The phenotypes included in this table are those with significant aggregate SGE (P < 0.05) and those for which the correlation ρ could be precisely estimated (i.e. aggregate SGE and aggregate DGE > 5%). The P value for aggregate is for being different from 0, that for ρ is for being different from 1.*

Correlation between SGE and DGE acting on the same phenotype

One mechanism underlying social effects is that of phenotypic "contagion" or "spread", whereby the phenotype of interest of a focal individual is influenced by the same phenotype of their social partners. In humans, cognitive susceptibility to depression, alcohol consumption, obesity, and educational attainment, only to name a few, have all been shown to be "contagious" or "spread" across college roommates, friends, spouses, or parent/offspring[3-5,13-17]. In contrast, few studies have considered more complex mechanisms whereby other traits of social partners influence the phenotype of interest. sgeGWAS, in principle, can discover such mechanisms if they exist. Before performing sgeGWAS, we assessed evidence of complex mechanisms of social effects by leveraging the parameter ρ of the variance components model used for variance decomposition.

This parameter captures the correlation between the SGE and DGE random effects (see Methods and Figure 1c), meaning that it estimates the similarity (in terms of loci and effect sizes) between SGE and DGE acting on the same trait. Simulations showed that the precision with which ρ can be estimated depends on the aggregate contribution of both SGE and DGE (Supplementary Figure 1), so we limited this analysis to 27 phenotypes for which both DGE and SGE explained more than 5% of phenotypic variation. The average value of ρ across these traits was 0.47, and for ten measures ρ was significantly different from one ($P < 0.05$, Table 1). ρ significantly different from one implies that different loci and/or loci with different effect sizes underlie DGE and SGE acting on the same phenotype, which rules out phenotypic contagion as the sole mechanism of social effects and indicates that other traits of cage mates mediate the social influence. Importantly, phenotypes such as depression and stress, which have been shown to "spread" across roommates and spouses[13,17], had ρ significantly different from 1 (Table 1), implying that traits of cage mates other than (or in addition to) depression or stress mediate social effects on depression and stress. In conclusion, our results suggest that complex mechanisms of social effects are common, which motivates the use of sgeGWAS to uncover them.

sgeGWAS and dgeGWAS of 170 phenotypes

To map SGE, we calculated the "social genotype" of a focal mouse as the sum of the reference allele dosages of its cage mates at the variant, and tested for association

between social genotype and phenotype. In order to avoid spurious associations, we accounted for background SGE, DGE and non-genetic effects using an extension of the variance components model used for variance decomposition; we also accounted for local DGE when testing local SGE and *vice versa* (See Methods). Indeed, we found that direct and social genotypes at a variant tended to be more correlated than permuted genotypes (Supplementary Figure 2a, 2b and 2c), as a result of each mouse serving both as focal individual and cage mate in the analysis (Supplementary Note). Using each individual as focal individual and social partner is a strategy that has been used before[10] as it maximises sample size when all individuals have been both phenotyped and genotyped, but the resulting genotypic correlations and their consequences had not been documented. We found that even small correlations (R^2 < 0.04) can lead to spurious SGE in the presence of a co-localised large-effect DGE (Supplementary Figure 2d). Conditioning on the direct genotypes at the locus tested avoided spurious associations (Supplementary Figure 2e). The impact on power of conditioning depended on whether a large-effect DGE co-localised with the tested SGE and on the correlation between direct and social genotypes (Supplementary Figure 3a-d). In the real data we found less significant genotypic correlations at genome-wide significant SGE and DGE associations, suggesting that conditioning may lead to “blind spots” where genetic effects are harder to detect (Supplementary Figure 3e).

sgeGWAS identified 21 genome-wide significant loci for 17 of the 170 phenotypes (per-phenotype FDR <10%, Figure 2 and Table 2). In comparison, dgeGWAS identified 118 genome-wide significant loci for 63 of the same 170 phenotypes (Figure 2 and Supplementary Table 2). There was no overlap between genome-wide significant SGE and DGE acting on the same phenotype, but variants at genome-wide significant SGE loci were enriched in small P values of DGE association with the same phenotype (Supplementary Figure 4). Together these results suggest a partially distinct basis for SGE and DGE (i.e. partially different loci and/or effect sizes), which is consistent with the conclusion from the analysis of the correlation parameter ρ.

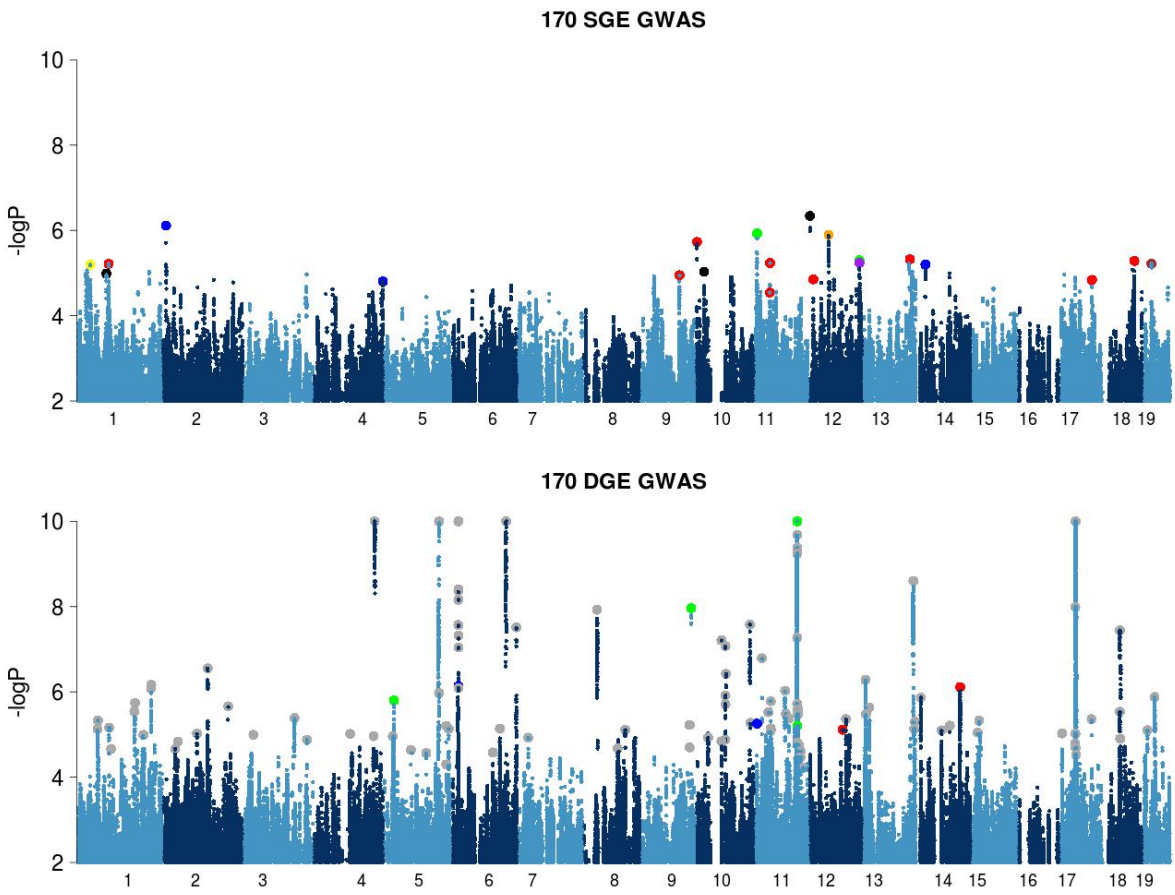

Figure 2 *Superimposed manhattan plots corresponding to 170 sgeGWAS (top panel) and 170 dgeGWAS of the same phenotypes (bottom panel). Lead variants for all genome-wide significant SGE and DGE loci are represented with a larger dot. In the SGE panel, each color corresponds to a class of phenotypes: behavioural (red), adult neurogenesis (black), immune (orange), haematological (yellow), blood biochemistry (blue), bone phenotypes (green), heart function (brown), and lung function (purple). In the DGE panel, a genome-wide significant locus is colored grey when the corresponding phenotype does not have a genome-wide significant SGE association; when the corresponding phenotype does have an SGE association, the same color is used as in the SGE panel. Data points with negative log P < 2 are not shown.*

Name	Chr	Pos (bp)	logP	% variance	Candidate genes
Haem.abs_neuts	1	35731852	5.2	2.1	Hs6st1 Uggt1 Neurl3
Neuro.Ki67_BWcorr	1	71339531	5	2.3	Abca12
PST.Immobility.First2min	1	76512651	5.2	1.7	Epha4
Bioch.Chloride_BWcorr	2	9524584	6.1	2	Gata3, Taf3, Atp5c1, Kin, Itih2, Itih5
Bioch.Calcium_BWcorr	4	156234293	4.8	1.6	Rnf223 Agrn Isg15 Perm1 Plekhn1 Klhl17 Noc2l Samd11 Vmn2r123 Samd11
PST.Immobility.Last4min	9	88497500	4.9	1.3	Ripply2 Cyb5r4 Mrap2 Cep162 Tbx18 Nt5e Snx14 Syncrip Zfp949 Trim43a Mthfsl
PAS.First5	10	3128239	5.7	1.6	H60c
Neuro.Ki67_BWcorr	10	19045006	5	2.4	Nhsl1 Hebp2 Arfgef3 Perp Tnfaip3
BMC.osteoporosis	11	7019042	5.9	1.5	Ddx56 Tmed4 Ogdh Zmiz2 Ppia H2afv Purb Myo1g Adcy1 Igfbp1 Igfbp3
Sleep.s12h_L_BWcorr	11	35487784	4.5	2	Slit3 Pank3 Wwc1 Rars Fbll1 Tenm2
Sleep.s12h_D_BWcorr	11	35909978	5.2	1.9	Slit3 Pank3 Wwc1 Rars Fbll1 Tenm2
Neuro.DCX_BWcorr	12	3176906	6.3	2.6	Rab10os
Sleep.VAR_1h_BWcorr	12	10841326	4.9	2	Pgk1-rs7
FACS.CD3posCD4pos	12	45005068	5.9	2.4	Nrcam Stxbp6
BMC.Kurt	12	113706627	5.3	1.3	Igh*
Hypoxia.f_NR_BWcorr	12	113742891	5.2	1.8	Ighv5-9-1
Sleep.s12h_D_BWcorr	13	106698960	5.3	1.8	Rnf180 Htr1a Olfr717-ps1 Dph3b-ps
Bioch.Calcium_BWcorr	14	21325786	5.2	1.8	Plau Vcl Ap3m1 Adk Kat6b Dupd1 Dusp13 Samd8 Vdac2 Comtd1
PST.Immobility.Last4min	17	71277261	4.8	1.2	Dlgap1 Tgif1 Myl12b Myl12a Myom1 Lpin2 Emilin2 Smchd1
Sleep.sDif_LD_BWcorr	18	71042115	5.3	2.4	Tcf4 Ccdc68 Rab27b Dynap Stard6 Poli Mbd2
Cardio.ECG.Tpeak_Tend_BWcorr	19	18645437	5.2	2.2	Carnmt1 Nmrk1 Ostf1 Trmp6

See Supplementary Table 1 for a description of the phenotypes

Igh* denotes a large number of Igh (immunogloblin hypervariable region) genes

Table 2 *Genome-wide significant SGE associations (per-phenotype FDR < 10%). Significance and proportion of phenotypic variance explained are indicated for each association, as well as candidate genes at each locus (see Methods).*

At five genome-wide significant SGE loci we identified a single candidate gene (Table 2, Supplementary Figure 5). *Abca12*, a gene known for its involvement in lipid transport and homeostasis in the skin[21], gave rise to SGE on a measure of adult neurogenesis in the hippocampus; *Epha4*, a signalling genes involved in neural system function, influenced cage mates' helplessness; *H60c*, a poorly characterised gene potentially involved in skin immunity[22], influenced locomotor activity of cage mates; *Pgk1-rs7*, a pseudogene of phosphoglycerate kinase-1, affected cage mates' sleep; finally, *Ighv5-9-1*, a variable region of the T cell receptor, influenced cage mates' response to hypoxia. The mechanism of action of these genes on the phenotypes of cage mates could not be inferred from what is known of their direct effects, which illustrates the potential for sgeGWAS to discover novel mechanisms of social effects but also shows the difficulty of translating sgeGWAS findings into biological insights, a difficulty commonly met after dgeGWAS.

Architecture of SGE and comparison with that of DGE

Despite being carried out on the same individuals and phenotypes, and in a perfectly analogous manner, sgeGWAS identified fewer genome-wide significant associations than dgeGWAS (21 associations for 17 phenotypes and 118 associations for 63 phenotypes respectively). As very few studies have mapped SGE and none have investigated the determinants of power for SGE, it was not clear whether we had different power to detect SGE and DGE associations. In order to get a better understanding of this issue, we simulated local SGE or DGE and calculated power to detect these associations. Briefly (see Methods), we considered random groups of three mice per cage, and simulated phenotypes arising from the sum of local genetic effects (DGE or SGE), polygenic effects (DGE and SGE), and environmental effects. For local SGE, we considered two alternative generative models, both consistent with the additive model used for sgeGWAS: an "additive" model in which the social allele of a mouse is the sum of the minor direct alleles of its two cage mates, and a

"proportional" model in which the social allele is the proportion of minor direct alleles across cage mates. For each type of local effect (DGE, additive SGE and proportional SGE), we considered variants with low, medium or high minor direct allele frequencies (MAF, defined as "traditionally" based on direct genotypes). For all three types of local effects we simulated the same allelic effect (but the definition of "allele" was different for different types of local effects).

Our simulations showed that power always increases with MAF (Figure 3a). For a given MAF, power was greater for additive SGE than for DGE, as a result of the larger number of alleles giving rise to SGE as compared to DGE (4 versus 2). Indeed, simulating SGE from a single cage mate led to the same power as for DGE (simulating SGE from a single cage mate is actually equivalent to simulating DGE) and simulating SGE from an increasing large number of cage mates under the additive model led to greater and greater power. In contrast, power to detect SGE simulated under the proportional model from two cage mates was lower than power to detect DGE. These results are all consistent with the fact that, for a given phenotype and sample size, power to detect an local effect (DGE or SGE) is determined by the phenotypic variance explained by the locus, which is equal to the product of the square of the allelic effect (set to a constant in these simulations) and the genotype variance. Noting MAF as p and number of cage mates as N, the genotype variance is $2p(1-p)$ for DGE, $2Np(1-p)$ for SGE under the additive model, and $2Np(1-p)/N^2$ for SGE under the proportional model. In conclusion, our simulations showed that power to detect local genetic effects is generally determined by allelic effect and genotype variance; the former depends on the definition of allele (direct, social additive or social proportional) and the latter on the number of cage mates (for SGE) as well as the definition of allele. Consequently, allelic effects and genotype variances are most useful to compare sgeGWAS results (so long as the same number of social partners and definition of social allele are used); SGE and DGE associations, in contrast, are best compared in terms of variance explained as this overcomes definition issues.

Accordingly, we report social allelic effects and genotype variances of genome-wide significant SGE associations for comparison with future sgeGWAS (Supplementary Figure 6) but focus here on comparing genome-wide significant SGE and DGE associations in terms of variance explained. This comparison yielded two main results: firstly, genome-wide significant SGE associations explained a maximum of 2.5% of phenotypic variance, while eleven genome-wide significant DGE

associations explained more than 5% of phenotypic variance (Figure 3b). This result shows that sgeGWAS is unlikely to uncover “low hanging fruits”. Secondly, SGE and DGE genome-wide significant associations explained a similar fraction of the corresponding aggregate variance (mean 32.5% and 32.1% respectively, Figure 3c and 3d), suggesting that a similar number of variants give rise to SGE and DGE.

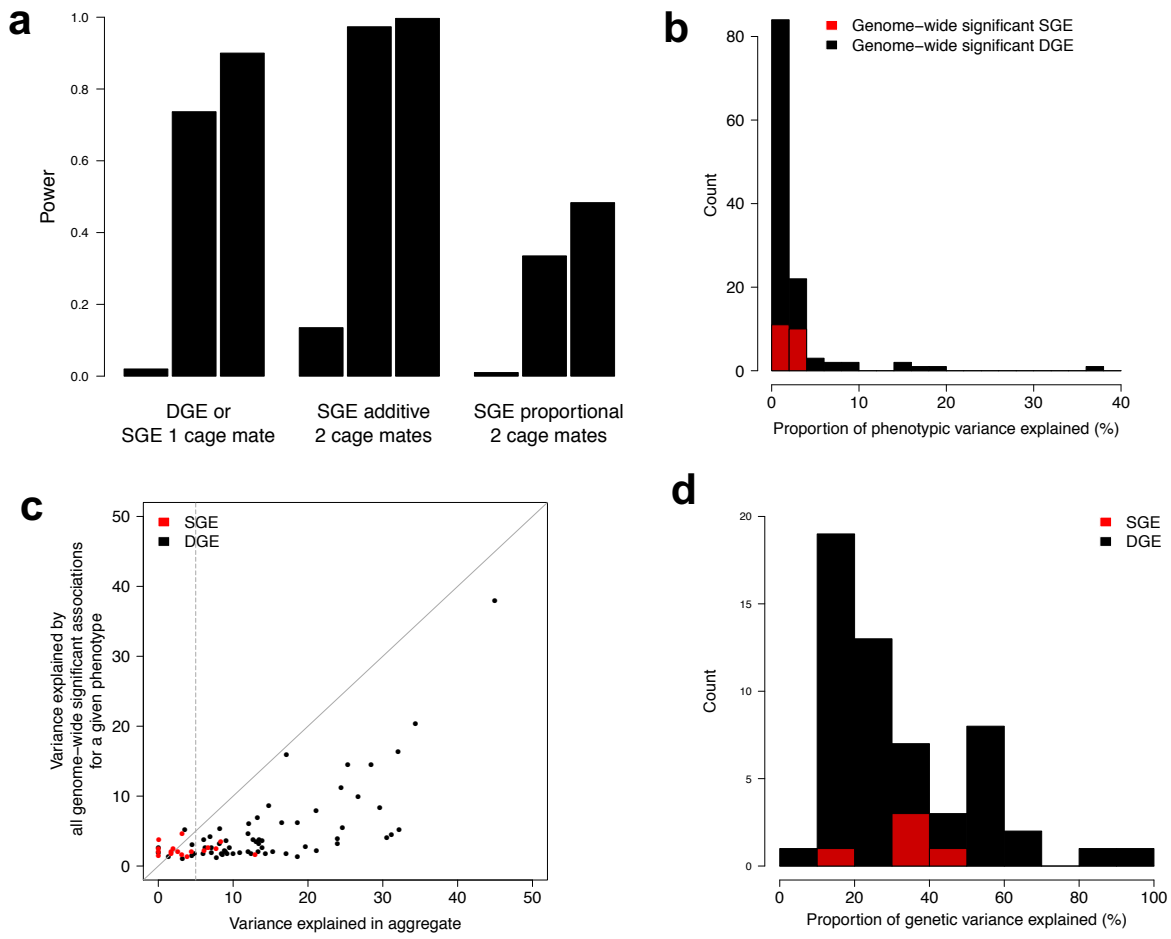

Figure 3 *Power to detect local SGE and DGE, and characterisation of the architecture of SGE and DGE. (a) Power to local genetic effects in simulations. Three types of local genetic effects were simulated: DGE (or, equivalently, SGE arising from a single cage mate), SGE arising from two cage mates under an additive model, and SGE arising from two cage mates under a proportional model. For each type of effect, results are shown (left to right) for variants with low MAF (MAF < 0.05), medium MAF (0.225<MAF<0.275) and high MAF (MAF>0.45) (MAF: minor allele frequency, defined as traditionally based on direct genotypes). (b) Histogram of the proportion of phenotypic variance explained by genome-wide significant SGE (red) and DGE (black) associations. (c) Comparison of the variance explained by social (red) and direct*

(black) genetic effects in aggregate (x axis) and the corresponding genome-wide significant associations (y axis). Each dot corresponds to a phenotype with a genome-wide significant association. A faint dashed line shows the 5% cutoff used to calculate the ration plotted in (d). (d) Histogram of the ratio between phenotypic variance explained by all genome-wide significant associations for a given trait and aggregate variance, for SGE (red) and DGE (black). Only phenotypes for which the aggregate variance was greater than 5% were considered for (d).

Discussion

Our study explored a promising approach, sgeGWAS, to uncover novel mechanisms of social effects. For the first time we carried out sgeGWAS in an outbred population. We analysed a broad range of biomedical phenotypes measured in laboratory mice and performed dgeGWAS on the same phenotypes so as to get general insights about SGE. Our main results are the following: first, SGE and DGE arose from different loci and/or loci with different effect sizes; secondly, SGE associations, in contrast with DGE associations, never explained a large proportion of phenotypic variance; finally, genome-wide significant SGE and DGE associations explained a similar fraction of the corresponding aggregate variance.

This study adds to an increasing body of evidence identifying SGE as an important component of phenotypic variation, and sheds light on an ever wider reach of social effects. Indeed, while previous work in laboratory mice has reported aggregate SGE between family members[6-8], we have evidence in this study of significant and substantial aggregate SGE between unrelated cage mates, which suggests that SGE may arise in any type of social relationship. This result is consistent with findings in other species including humans[3-5].

Furthermore, we found that SGE affected a broad range of phenotypes, some of which are known to be socially affected (e.g. mood and immune status) while others are not (e.g. LDL levels, platelet size, rate of wound healing). The literature on social effects has largely focused on phenotypes for which the mechanism of phenotypic "contagion" is conceivable (e.g. mood[13], alcohol consumption[14,15], but not LDL levels or wound healing), because in the absence of such prior one would need to measure a very large number of traits of social partners and test whether they affect the phenotype of interest, which is impractical. Using SGE we were able to overcome this

limitation and test social effects on each of the 170 phenotypes in the dataset, independently of the likelihood of phenotypic contagion. Doing so, we uncovered social effects on phenotypes not known to be socially affected. By comparing SGE and DGE acting on the same trait we further demonstrated that phenotypic contagion in many cases is not sufficient to explain social effects. Thus, our results empirically establish a need for social studies to consider more complex mechanisms of social effects, and suggest SGE may be used as a tool to do so.

It is important to note that SGE can and have been used in a more 'targeted' approach, to test whether a specific trait of social partners affects the phenotype of interest. For example, three recent studies in humans[3-5] have investigated whether educational attainment (EA), height (HT) and body-mass index (BMI) of social partners (friends or parents) affect EA of focal individuals. These studies used polygenic scores derived from large pre-existing dgeGWAS of EA, HT and BMI to correlate the polygenic score of social partners with EA of focal individuals. Thus, they relied on aggregate – but not genome-wide - SGE to test whether specific traits of social partners affect the phenotype of interest. The goal of sgeGWAS is quite different, namely identifying novel, unsuspected mechanisms of social effects.

Another appeal of sgeGWAS is that it may help characterise the architecture of SGE, which we know from DGE studies to be important[23]. We found that, in this population, individual SGE associations never explained a large fraction of phenotypic variance, which contrasts with DGE associations explaining up to 40% of phenotypic variance. As effect size is key to identify genes underlying GWAS loci[24], this greatly reduced our ability to identify novel genes and mechanisms of social effects. More generally, this result suggests sgeGWAS will yield few "low hanging fruits" and require large sample sizes as well as optimised designs. Our results also suggested that SGE and DGE likely arose from a similar number of variants. Information about the levels of polygenicity of genetic effects can help develop models for their mode of action[25]. Here we reasoned that the level of polygenicity of SGE being a function of the number of traits of cage mates that mediate social effects and their respective DGE polygenicity, a similar level of polygenicity between SGE and DGE was consistent with two mechanistic scenarios: SGE arising from a small number of traits of cage mates or SGE arising from multiple traits of cage mates with a simpler DGE architecture than the phenotypes included in this dataset. Such traits could be "social phenotypes", such as aggression or pheromone levels, because they were notably missing from this

dataset and the DGE architecture of "social phenotypes" is poorly understood compared with biomedical traits; thus, it could be simples. However, our inference on polygenicity levels is based on a very small number of phenotypes for SGE (five), so additional sgeGWAS are required to further explore this question.

Our study made several important methodological contributions that will help design, perform and interpret sgeGWAS, particularly in outbred populations where both DGE and SGE contribute to phenotypic variation. Specifically, we presented a model to account for (background) polygenic DGE and SGE in sgeGWAS. We also described the determinants of power for SGE, and finally shed some light on an important issue arising when the same individuals are used as focal individuals and social partners, namely correlations between direct and social genotypes. These genotypic correlations will result in spurious SGE associations unless accounted for by conditioning on direct genotypes. Our results suggest this strategy may unfortunately lead to true SGE associations being missed. Correlations between direct and social genotypes may arise for different reasons in other datasets, notably when focal individuals and social partners are related, or as a result of direct assortments (e.g. assortative mating[26,27], homophily between friends[3]). Accounting for these correlations will be key to the interpret the results of sgeGWAS correctly.

Our study sheds light on a promising approach, sgeGWAS, to dissect the mechanisms of social effects. This information is important to understand the evolution of social species and for applied goals such as improved welfare and healthcare[23].

Methods

Phenotypes and experimental variables

Phenotypes and experimental variables (covariates) for 1,934 Crl:CFW(SW)-US_P08 (CFW) mice were retrieved from http://wp.cs.ucl.ac.uk/outbredmice/. We normalized each phenotype using the boxcox function (MASS package[28]) in R, and excluded phenotypes that could not be normalised satisfactorily (lambda outside of -2 to 2 interval). The subset of covariates used for each phenotype is indicated in Supplementary Table 1. Because data for some phenotypes were missing for some

mice, the sample size varied. The sample size for each phenotype after all filtering (see below) is indicated in Supplementary Table 1.

Caging information

Mice were four to seven weeks old when they arrived at the phenotyping facility. They were grouped with their cage mates and then spent nine to twelve weeks undisturbed in quarantine. They spent a further four weeks together during phenotyping. Males were always housed with males and females with females.

Cage assignments were not included in the publicly available dataset but were provided by the authors upon request and are now provided in Supplementary Table 3. Cage assignments were recorded at eleven time points throughout the study and showed that a few mice were taken out of their original cages and singly housed, presumably because they were too aggressive to their cage mates. When this happened, we excluded all the mice in that cage from the analysis. We also excluded cages where some of the mice were "genetically close" (as defined below) to many other mice. Finally, we only retained cages with exactly three mice per cage. Although from the sleep test on all mice were singly housed, we still investigated "persistent" SGE on sleep and tissue phenotypes (persistence over one day for sleep phenotypes and over one week maximum for tissue measures).

Genome-wide genotypes

From http://wp.cs.ucl.ac.uk/outbredmice/ we retrieved both allele dosages for 7 million variants and allele dosages for a subset of 353,697 high quality, LD-pruned variants (as described in Nicod et al.[19]). We used high quality, LD-pruned variants for all analyses but the identification of candidate genes at SGE loci (see below), for which we used the full set of variants.

Genetic relatedness matrix (GRM) and exclusion of "genetically close" mice

The genetic relatedness matrix was calculated as the cross-product of the dosage matrix after standardizing the dosages for each variant to mean 0 and variance 1.

We excluded some mice and all their cage mates based on GRM values as follows: we defined a "close pair" of mice as having a GRM value greater than 0.3 (based on the histogram of all GRM values). 199 mice in 145 cages were involved in such close pairs. Excluding all mice from these 145 cages plus their cage mates would have resulted in excluding 435 mice out of a total of 1,812. As this would have reduced power too much for sgeGWAS and dgeGWAS, we only excluded mice from cages involved in 4 or more close pairs (19 cages, 57 mice).

Variance decomposition

The same method as described in details in Baud et al.[6] was used. Briefly, the model used was:

$$y_f = X_f \underline{b} + a_{D,f} + e_{D,f} + Z_f \underline{a_S} + Z_f \underline{e_S} + W_f \underline{c} + Z_f G_S b_S \quad (0)$$

y_f is the phenotypic value of the focal mouse f, X_f is a row of the matrix X of covariate values and b a column vector of corresponding estimated coefficients. $\underline{a_{D,f}}$ is the additive direct genetic effects (DGE) of f. Z_f is a row of the matrix Z that indicates cage mates (importantly $Z_{i,i} = 0$) and $\underline{a_S}$ the column vector of additive social genetic effects (SGE). $\underline{e_D}$ refers to direct environmental effects and $\underline{e_S}$ to social environmental effects. W_f is a row of the matrix W that indicates cage assignment and c the column vector of cage effects.

The joint distribution of all random effects is defined as:

$$\begin{bmatrix} \underline{a_D} \\ \underline{a_S} \\ \underline{e_D} \\ \underline{e_S} \\ \underline{c} \end{bmatrix} \sim \mathrm{MVN}\left(0, \begin{bmatrix} \sigma^2_{A_D}\mathrm{A} & \sigma_{A_{DS}}\mathrm{A} & 0 & 0 & 0 \\ \sigma_{A_{DS}}\mathrm{A}^T & \sigma^2_{A_S}\mathrm{A} & 0 & 0 & 0 \\ 0 & 0 & \sigma^2_{E_D}\mathrm{I} & \sigma_{E_{DS}}\mathrm{I} & 0 \\ 0 & 0 & \sigma_{E_{DS}}\mathrm{I}^T & \sigma^2_{E_S}\mathrm{I} & 0 \\ 0 & 0 & 0 & 0 & \sigma^2_C\mathrm{I} \end{bmatrix}\right)$$

where A is the GRM and I the identity matrix.

The phenotypic covariance is:

$$C_{i,j} = cov(y_i, y_j)$$
$$= \sigma^2_{A_D} A_{i,j} + \sigma_{A_{DS}} + \sigma^2_{A_S}(ZAZ^T)_{i,j} + \sigma^2_{E_D} I_{i,j} + \sigma_{E_{DS}}\{(IZ^T)_{i,j}$$
$$+ (ZI^T)_{i,j}\} + \sigma^2_{E_S}(ZIZ^T)_{i,j} + \sigma^2_C (WIW^T)_{i,j}$$

The variances explained by DGE and SGE were calculated respectively as $sampleVar\left(\sigma^2_{A_D}\, A\right)$ / $sampleVar(C)$ and $sampleVar\left(\sigma^2_{A_S}(ZAZ^T)\right)$ / $sampleVar(C)$ where $sampleVar$ is the sample variance of the corresponding covariance matrix: suppose that we have a vector $\underline{x}$ of random variables with covariance matrix M, the sample variance of M is calculated as

$$sampleVar(M) = \frac{Tr(PMP)}{n-1}$$

Tr denotes the trace, n is the sample size, and $P = I - \frac{11'}{n}$ [29,30].

For those phenotypes where body weight was included as a covariate, we checked that this did not lead to systematically increased (or decreased) estimates of the aggregate contribution of SGE (collider bias).

Significance of variance components was assessed using a two-degree of freedom log likelihood ratio (LLR) test (i.e., the test statistics was assumed to follow a two-degree of freedom chi2 distribution under the null). Note that this testing procedure is conservative.

Correlation between DGE and SGE

The correlation ρ between $\underline{a_D}$ and $\underline{a_S}$ was calculated as:

$$\rho = \frac{\sigma_{A_{DS}}}{\sigma_{A_D} \times \sigma_{A_S}}$$

ρ reflects the correlation between SGE and DGE similarly to how "traditional" genetic correlations measure the correlation between DGE on two traits; ρ can actually be interpreted as the correlation between effect sizes of DGE on the mediating trait(s) of cage mates and DGE on the phenotype of interest.
We tested whether ρ was significantly different from 1 using a one-degree of freedom LLR test.

Simulations 1: for Supplementary_Figure1.

Phenotypes were simulated based on the genotypes and cage relationships of the full set of 1,812 mice. Phenotypes were drawn from model (0) with the following variances: $\sigma^2_{A_D} = 15$, $\sigma^2_{A_S} = 8$, $\rho_{A_{DS}} = 0.47$, $\sigma^2_{E_D} = 22$, $\sigma^2_{E_S} = 16$, $\rho_{E_{DS}} = -0.97$, $\sigma^2_C = 26$. These variances correspond to the median value of estimates across traits with aggregate SGE and DGE > 5%. After building the phenotypic covariance matrix, the sample variance of the simulations was calculated and used to calculate "realised" simulation parameters from the "target" parameters above. The realised parameters were used for comparison with the parameters estimated from the simulations.

Definition of "social genotype" for sgeGWAS

In the sgeGWAS, we assumed additive effects across cage mates and calculated the "social genotype" of a mouse as the sum of the reference allele dosages of its cage mates.

Correlation between direct and social genotypes at a variant

Spearman's rank correlation coefficient was used. We tested whether the correlation was different from 0 using the function cor.test in the R package stats[31].

Models used for sgeGWAS and dgeGWAS

To test SGE of a particular variant, we compared the following two models:

$$y_f = X_f\underline{b} + a_{D,f} + e_{D,f} + Z_f\underline{a_S} + Z_f\underline{e_S} + W_f\underline{c} + G_f b_D \qquad \text{(1, null)}$$

$$y_f = X_f\underline{b} + a_{D,f} + e_{D,f} + Z_f\underline{a_S} + Z_f\underline{e_S} + W_f\underline{c} + G_f b_D + Z_f G b_S \qquad \text{(2, alternative)}$$

Here, G is the vector of direct genotypes at the tested variant, b_D the estimated coefficient for local DGE and b_S the estimated coefficient for local SGE.
The models were fitted using LIMIX[32,33] with the covariance of the model estimated only once per phenotype, in the model with no local genetic effect (model 0).

The significance of local SGE was calculated by comparing models (1) and (2) with a 1-degree of freedom LLR test.
We refer to the inclusion of $G_f b_D$ in model (1) as "conditioning".

dgeGWAS was carried out similarly, by comparing model (3) and model (2) below:

$$y_f = X_f \underline{b} + a_{D,f} + e_{D,f} + Z_f \underline{a_S} + Z_f \underline{e_S} + W_f \underline{c} + Z_f G b_S \qquad \text{(3, null)}$$

We refer to the inclusion of $Z_f G b_S$ in model (3) as "conditioning".

Identification of genome-wide significant associations

Following Nicod et al.[19], for each phenotype and for each type of genetic effect (social and direct), we ran 100 "permuted GWAS" by permuting the rows of the matrix of social (respectively direct) genotypes, and testing each variant at a time using the permuted genotypes together with the un-permuted phenotypes, covariates, GRM and matrix of direct (respectively social) genotypes (for conditioning). For each permutation we then compiled a list of loci that would be significant at a nominal P value of 0.01. Using the un-permuted data, we similarly compiled a list of loci that would be significantly associated at a nominal P value of 0.01. Ordering the latter in order of decreasing significance and going down the list, we calculated for each locus an associated FDR until the FDR was above 10%. For a given P value x, the FDR was calculated as:

$$FDR(x) = \frac{\#\ loci\ with\ P < x\ in\ permuted\ data}{100 \times \#\ loci\ with\ P < x\ in\ unpermuted\ data}$$

We report only those loci whose P value corresponds to an FDR < 10%.
This strategy controls the per-phenotype FDR; it does not mean that the study-wide FDR is <10% (the study-wide FDR cannot be calculated as the false positive rate is not defined for those phenotypes where the 10% per-phenotype FDR was never achieved).

Definition of candidate genes at associated loci

At each significantly associated locus we defined a 4Mb window centered on the lead variant, identified all variants in this window based on the full set of 7M variants, and

reran the sgeGWAS with those variants. We then defined candidate genes based on the association profile in the 4Mb window.

Variance explained by genome-wide significant SGE and DGE associations

The variance explained by genome-wide significant SGE and DGE associations was estimated using model (2) and calculated, respectively, as:

$$\frac{var(Gb_D)}{\sum var(X_c b_c) + var(Gb_D) + var(ZGb_S) + sampleVar(C)}$$

and

$$\frac{var(ZGb_S)}{\sum var(X_c b_c) + var(Gb_D) + var(ZGb_S) + sampleVar(C)}$$

Simulations 2: for Supplementary Figure 2d and 2e.

Phenotypes were simulated based on the genotypes and cage relationships of the full set of 1,812 mice. Phenotypes were simulated as the sum of random effects and local DGE (from model (1)), with the following parameters: $\sigma^2_{A_D} = 5$ or 20, $\sigma^2_{A_S}$ = 5 or 20, $\rho_{A_{DS}}$= 0.5, $\sigma^2_{E_D}$= 30, $\sigma^2_{E_S}$= 30, $\rho_{E_{DS}}$ = -0.97, σ^2_C = 25. The values for $\rho_{A_{DS}}$, $\sigma^2_{E_D}$, $\sigma^2_{E_S}$, $\rho_{E_{DS}}$, and σ^2_C were close to the median of the corresponding estimates from the real data. $\sigma^2_{A_D} = 5$ and $\sigma^2_{A_S} = 5$ correspond to low polygenic effects in the real data, and $\sigma^2_{A_D} = 20$ and $\sigma^2_{A_S} = 20$ correspond to high polygenic effects in the real data. We simulated local DGE at variants where direct and social genotypes were either lowly correlated (Spearman correlation negative log P value < 0.05) or more highly correlated (negative log P value > 2), and simulated variances of 0, 5, 20 or 50.

The results we show in Supplementary Figure 2d and 2e are based on a subset of simulations: $\sigma^2_{A_D} = 20$ and $\sigma^2_{A_S} = 20$ and local DGE variance of 20.

Simulations 3: for Supplementary Figure 3a-d, and Figure 3a.

Phenotypes were simulated based on the real genotypes but random cages for a random subset of 1,800 mice (in order to be able to draw full cages of 2, 3, 4, 5, or 6 mice). Phenotypes were simulated as the sum of random effects, local DGE and local SGE (model (2)) with the following parameters: $\sigma^2_{A_D} = 17$, $\sigma^2_{A_S} = 17$, $\rho_{A_{DS}} = 0.65$, $\sigma^2_{E_D} = 19$, $\sigma^2_{E_S} = 15$, $\rho_{E_{DS}} = -0.8$, $\sigma^2_C = 25$. Those values correspond to the median estimates for phenotypes with aggregate SGE and DGE > 0.1.

We simulated local SGE and DGE at variants where direct and social genotypes were either lowly correlated (Spearman correlation negative log P value < 0.05) or more highly correlated (Spearman correlation negative log P value > 0.2), and had with low MAF (MAF < 0.05), medium MAF (0.225<MAF<0.275) or high MAF (MAF>0.45). We simulated local DGE with an allelic effect of 0 or 1 (1 corresponds to a large effect in the real data) and simulated local SGE under the additive or the proportional model, in all cases with an allelic effect of 0.2 (similar to the average allelic effect estimated under the additive model in the real data).

The results we show in Supplementary Figure 3a-d are based on a subset of simulations with group size 3 and are averaged across low, medium and high MAF. Power was calculated at a genome-wide significance threshold of negative log P 5, which is similar to the significance of associations detected at FDR < 10%.

The results we show in Figure 3a are based on a subset of simulations with group size 2 and 3, no local DGE, and averaged across high and low genotypic correlations. Power was also calculated at a genome-wide significance threshold of negative log P 5.

Scripts used in this study

All the scripts used in this study are available from http://github.com/limix/SGE.
LIMIX can be downloaded from http://github.com/limix/limix.

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References

1. Bergsma, R., Kanis, E., Knol, E.F. & Bijma, P. The contribution of social effects to heritable variation in finishing traits of domestic pigs (Sus scrofa). *Genetics* **178**, 1559–1570 (2008).
2. Wolf, J.B., Brodie III, E.D., Cheverud, J.M., Moore, A.J. & Wade, M.J. Evolutionary consequences of indirect genetic effects. *Trends in ecology & evolution* **13**, 64–69 (1998).
3. Domingue, B.W. *et al.* The social genome of friends and schoolmates in the National Longitudinal Study of Adolescent to Adult Health. *Proceedings of the National Academy of Sciences*, 201711803 (2018).
4. Kong, A. *et al.* The nature of nurture: Effects of parental genotypes. *Science* **359**, 424–428 (2018).
5. Bates, T.C. *et al.* The Nature of Nurture: Using a Virtual-Parent Design to Test Parenting Effects on Childrens Educational Attainment in Genotyped Families. *Twin Research and Human Genetics*, 1–11 (2018).
6. Baud, A. *et al.* Genetic variation in the social environment contributes to health and disease. *PLoS genetics* **13**, e1006498 (2017).
7. Ashbrook, D.G., Gini, B. & Hager, R. Genetic variation in offspring indirectly influences the quality of maternal behaviour in mice. *Elife* **4**, 11814 (2015).
8. Ashbrook, D.G., Sharmin, N. & Hager, R. Offspring genes indirectly influence sibling and maternal behavioural strategies over resource share. in *Proc. R. Soc. B* Vol. 284 20171059 (The Royal Society, 2017).
9. Visscher, P.M., Brown, M.A., McCarthy, M.I. & Yang, J. Five years of GWAS discovery. *The American Journal of Human Genetics* **90**, 7–24 (2012).
10. Biscarini, F. *et al.* Across-line SNP association study for direct and associative effects on feather damage in laying hens. *Behavior genetics* **40**, 715–727 (2010).
11. Mutic, J.J. & Wolf, J.B. Indirect genetic effects from ecological interactions in Arabidopsis thaliana. *Molecular ecology* **16**, 2371–2381 (2007).
12. Bailey, N.W. & Hoskins, J.L. Detecting cryptic indirect genetic effects. *Evolution* **68**, 1871–1882 (2014).
13. Haeffel, G.J. & Hames, J.L. Cognitive Vulnerability to Depression Can Be Contagious. *Clinical Psychological Science* **2**, 75–85 (2014).
14. Rosenquist, J.N., Murabito, J., Fowler, J.H. & Christakis, N.A. The spread of alcohol consumption behavior in a large social network. *Annals of internal medicine* **152**, 426–433 (2010).

15. Grant, J.D. *et al.* Spousal concordance for alcohol dependence: evidence for assortative mating or spousal interaction effects? *Alcoholism: Clinical and Experimental Research* **31**, 717–728 (2007).
16. Christakis, N.A. & Fowler, J.H. The spread of obesity in a large social network over 32 years. *New England journal of medicine* **357**, 370–379 (2007).
17. Bolger, N., DeLongis, A., Kessler, R.C. & Wethington, E. The contagion of stress across multiple roles. *Journal of Marriage and the Family*, 175–183 (1989).
18. Yalcin, B. *et al.* Commercially available outbred mice for genome-wide association studies. *PLoS genetics* **6**, e1001085 (2010).
19. Nicod, J. *et al.* Genome-wide association of multiple complex traits in outbred mice by ultra-low-coverage sequencing. *Nature genetics* **48**, 912 (2016).
20. Davies, R.W., Flint, J., Myers, S. & Mott, R. Rapid genotype imputation from sequence without reference panels. *Nature genetics* **48**, 965 (2016).
21. Smyth, I. *et al.* A mouse model of harlequin ichthyosis delineates a key role for Abca12 in lipid homeostasis. *PLoS genetics* **4**, e1000192 (2008).
22. Yoshida, S. *et al.* Involvement of an NKG2D ligand H60c in epidermal dendritic T cell-mediated wound repair. *The Journal of Immunology* **188**, 3972–3979 (2012).
23. Timpson, N.J., Greenwood, C.M., Soranzo, N., Lawson, D.J. & Richards, J.B. Genetic architecture: the shape of the genetic contribution to human traits and disease. *Nature Reviews Genetics* **19**, 110 (2018).
24. Flint, J., Valdar, W., Shifman, S. & Mott, R. Strategies for mapping and cloning quantitative trait genes in rodents. *Nature Reviews Genetics* **6**, 271 (2005).
25. Boyle, E.A., Li, Y.I. & Pritchard, J.K. An expanded view of complex traits: from polygenic to omnigenic. *Cell* **169**, 1177–1186 (2017).
26. Domingue, B.W., Fletcher, J., Conley, D. & Boardman, J.D. Genetic and educational assortative mating among US adults. *Proceedings of the National Academy of Sciences* **111**, 7996–8000 (2014).
27. Robinson, M.R. *et al.* Genetic evidence of assortative mating in humans. *Nature Human Behaviour* **1**, 0016 (2017).
28. Venerables, W. & Ripley, B. Modern applied statistics with S. (new york: Springer, 2002).
29. Kang, H.M. *et al.* Variance component model to account for sample structure in genome-wide association studies. *Nature genetics* **42**, 348–354 (2010).
30. Searle, S.R. *Matrix algebra useful for statistics*, (Wiley, 1982).
31. Best, D. & Roberts, D. Algorithm AS 89: the upper tail probabilities of Spearmans rho. *Journal of the Royal Statistical Society. Series C (Applied Statistics)* **24**, 377–379 (1975).

32. Lippert, C., Casale, F., Rakitsch, B. & Stegle, O. LIMIX: genetic analysis of multiple traits. (2014).

33. Casale, F.P., Rakitsch, B., Lippert, C. & Stegle, O. Efficient set tests for the genetic analysis of correlated traits. *Nature methods* **12**, 755 (2015).