Comparative architectures of direct and social genetic effects from the genome-wide association study of 170 phenotypes in outbred 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 can arise when two individuals interact and heritable traits of one influence the phenotype of the other. To better understand how SGE arise from individual genetic loci, we have derived statistical strategies for the joint analysis of SGE and traditional direct genetic effects (DGE, effects of an individual's genotypes on its own phenotype), which we applied to a dataset of 170 behavioural, physiological and morphological phenotypes measured in 1,812 outbred laboratory mice. Genome-wide association study of SGE and DGE identified 24 and 120 genome-wide significant loci respectively (FDR < 10%). We found no overlap between genome-wide significant SGE and DGE loci acting on the same phenotype, and a moderate correlation between SGE and DGE genome-wide, demonstrating that sgeGWAS has the potential to identify novel genetic loci contributing to inter-individual differences. We focused on two such loci to illustrate how individual SGE loci may help identify traits of social partners mediating social effects. Finally, we contrasted the effect sizes of genome-wide significant SGE and

DGE loci and found smaller effect sizes for the former: SGE associations explained a maximum of 2.5% of phenotypic variance when eleven genome-wide significant DGE associations explained more than 5% of phenotypic variance. Our results and the presented methods will guide the design and analysis of future sgeGWAS.

Main text

Introduction

Social interactions between two individuals can result in the phenotype of one individual being affected by genotypes of the other. Such effects arise when the phenotype of a focal individual is influenced by heritable traits of their social partner (Figure 1a) and are called social genetic effects (SGE) or indirect genetic effects[1-4].

SGE have been shown to contribute significantly and substantially to phenotypic variation in livestock, wild animals, plants and laboratory model organisms[5]. In laboratory mice, SGE have been found to affect behavioural, physiological, and morphological traits, including phenotypes not *a priori* expected to be socially affected such as wound healing[6-9]. In humans, effects of non-transmitted parental alleles have been detected on offspring's educational attainment[10-12]. Thus, SGE are an important component of the genotype to phenotype path.

In addition, SGE may be used as a tool to investigate social effects, in particular identify traits of social partners affecting a phenotype of interest, measured in focal individuals. Indeed, identifying individual variants of social partners associated with the phenotype of interest, through genome-wide association study of SGE (sgeGWAS), could provide new insights into the pathways of social effects in a similar manner as GWAS of "traditional" direct genetic effects (DGE, effects of an individual's genotypes on its own phenotype) has provided insights into the "within-body" pathways of phenotypic variation[13]. However, robust methods to perform sgeGWAS are not established and such experiments will require information on the architecture of SGE[14].

The architecture of SGE is largely unknown, as few studies have identified genomic loci that influence phenotypes of social partners. Quasi-Mendelian SGE

exist[15,16] but candidate gene studies and GWAS of SGE have revealed oligo- or polygenic architectures for a larger number of phenotypes[7,8,15-18]. Furthermore, it is currently unknown to what extent SGE loci overlap with DGE loci and how much phenotypic variance individual SGE loci explain. To address these questions and identify loci underlying SGE, we leveraged an existing dataset of 170 behavioural, physiological and morphological phenotypes measured in outbred "CFW" laboratory mice[19,20] and studied both SGE and DGE acting on each phenotype (Figure 1b and 1c). Our results and the methods we detail and validate will inform the design and analysis of future sgeGWAS carried out in outbred populations with any levels of relatedness and group sizes.

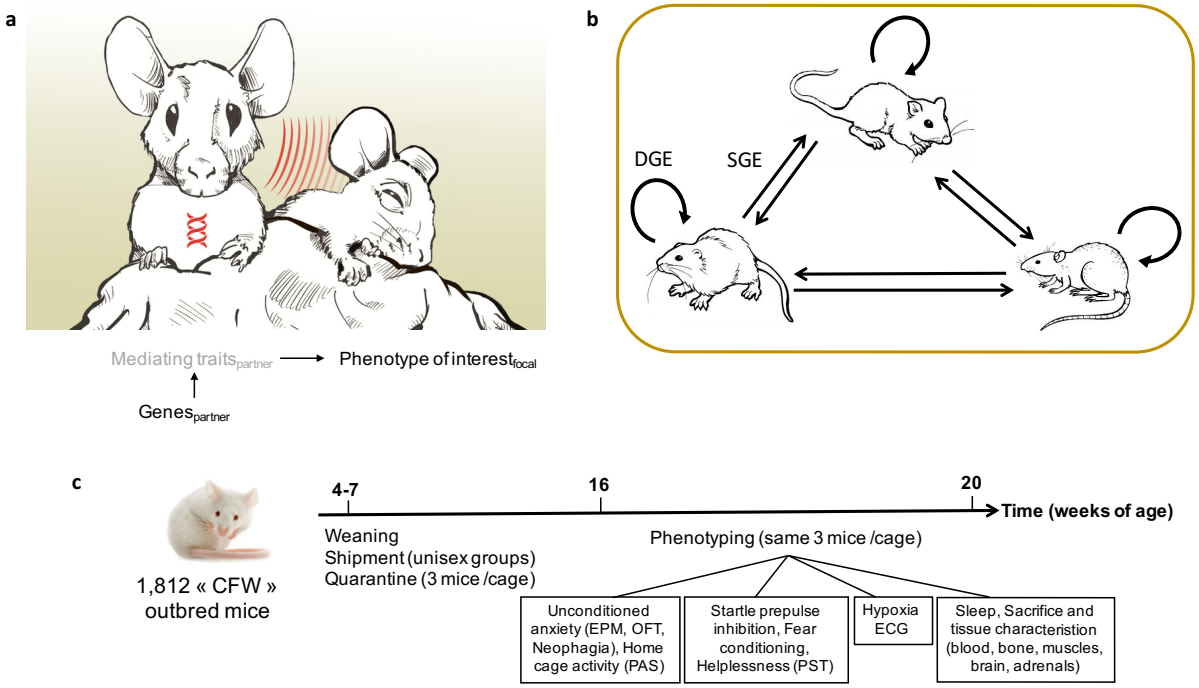


Figure 1 *Illustration of social genetic effects (SGE), definition of specific terms used throughout the manuscript, and experimental design. (a) SGE arise when two individuals interact and heritable traits of one influence the phenotype of the other. In other words, genotypes of the "social partner" influence - through "mediating traits" - the "phenotype of interest", measured in the "focal individual". Importantly, these mediating traits do not need to be measured or known. (b) 1,812 "CFW" outbred mice were housed in groups of 3 mice per cage. SGE and DGE contributed by each mouse were modelled, such that each mouse served as both focal individual and cage mate*

in our analyses. (c) Experimental design. The experimental paradigms used to collect the 170 phenotypes are listed.

Results

Genome-wide genotypes (both LD-pruned and non pruned, see Methods) and phenotypes for 2,073 CFW mice were available from Nicod et al.[19] and Davies et al.[20]. Males were always housed with males and females with females, and mice interacted in their cages for a period of at least nine weeks before phenotyping (Figure 1c). We only kept mice that had the same two cage mates over the course of the experiment (1,869 total). Furthermore, we excluded 57 mice that formed genetic substructures so that the remaining 1,812 mice were as equally related as possible while retaining as large a sample size as possible (see Methods).

Aggregate contribution of SGE

Initially we estimated the aggregate contribution of SGE (i.e. the sum of SGE across the genome) to all 170 phenotypes in the dataset, which include phenotypes that have never before been considered in a study of SGE (muscles and bones characteristics, response to hypoxia, electrocardiogram, and sleep). We used a random effect model for SGE and DGE as in Baud et al.[9] and found that SGE, in aggregate, explained up to 22% (+/- 6%) of variation in serum LDL levels and an average of 11% across 9 phenotypes with significant aggregate SGE (FDR < 10%, Supplementary Table 1). Phenotypes significantly affected by SGE included behavioural (helplessness), physiological (serum LDL cholesterol, healing from an ear punch, blood eosinophils, serum alpha-amylase concentration, blood platelets, acute hypoxic response), and morphological (weight of adrenal glands) traits. Importantly, among the phenotypes significantly affected by SGE were both phenotypes known or expected to be affected by the social environment, namely helplessness (a murine model for depression), blood eosinophils (a measure of immunity) and weight of adrenal glands (a measure associated with stress), and phenotypes less expected to be socially affected, such as LDL levels, response to hypoxia, and healing from an ear punch. Notably, the significant and substantial SGE detected in this population for healing from an ear punch ($P = 0.00018$, variance explained by SGE in aggregate = 17 +/-4%) constitute

the third independent line of evidence that SGE affect wound healing in laboratory mice[9]. Altogether, our results demonstrate that SGE may affect a broad range of phenotypes including traits not *a priori* expected to be socially affected, which is consistent with prior evaluation of SGE in other populations[9].

Correlation between SGE and DGE loci acting on the same phenotype

The polygenic model used for variance decomposition fits a correlation coefficient ρ (See Methods) that quantifies the extent to which SGE and DGE acting on the same phenotype arise from similar loci. We stress that, in this section and throughout the manuscript, we compare SGE and DGE *acting on the same phenotype* (but do this for all phenotypes). Using simulations, we observed that ρ can be accurately estimated, provided the aggregate contribution of SGE and DGE is sufficiently large (Supplementary Figure 1). Consequently, we limited the analysis of ρ to 28 phenotypes for which both aggregate SGE and aggregate DGE explained at least 5% of phenotypic variation. The SGE-DGE correlation coefficient ρ varied between -0.28 and 1 across these traits, with an average of 0.42 (Figure 2 and Supplementary Table 1). For 10 out of the 28 phenotypes ρ was significantly different from zero (nominal $P < 0.05$, Figure 2 and Supplementary Table 1), indicating that loci exist that influence both the phenotype of the focal animal (DGE) and the same phenotype of cage mates (SGE). Strong evidence for shared SGE and DGE loci ($\rho \neq 0$ at Bonferroni-corrected $P < 0.05$) was observed for healing from an ear punch, weight of adrenal glands, serum LDL cholesterol levels, and mean platelet volume.

We also evaluated evidence that $|\rho|$ was different from one (i.e. ρ different from one and minus one) in order to empirically evaluate the widely-influential model of "phenotypic contagion". Phenotypic contagion or "spread" is a model for social effects whereby the phenotype of interest of a focal individual is affected by the same phenotype of their social partners. In humans, cognitive susceptibility to depression, alcohol consumption, stress, obesity and educational attainment, for example, have been suggested to be "contagious" or "spread" across college roommates, spouses, friends, or parent/offspring[10-12,21-25]. As a result, phenotypic contagion has shaped the way we think about social effects: for example, phenotypes unlikely to spread have been used to cast doubt on social network effects[26]. Here we leveraged the parameter ρ to test whether phenotypic contagion was sufficient to account for SGE: under a

model of pure phenotypic contagion, |ρ| is expected to be equal to one; on the contrary, if traits of social partners other than the phenotype of interest mediate social effects, |ρ| is expected to be different from one. We found that |ρ| was significantly different from one (nominal P < 0.05) for 10 out of 28 phenotypes (Figure 2 and Supplementary Table 1), with strong evidence (P < 0.05 after Bonferroni correction) for helplessness and healing from an ear punch. Our results empirically demonstrate that traits of social partners other than the phenotype of interest may be important, even when some level of contagion is likely, and motivate the use of sgeGWAS to uncover such traits.

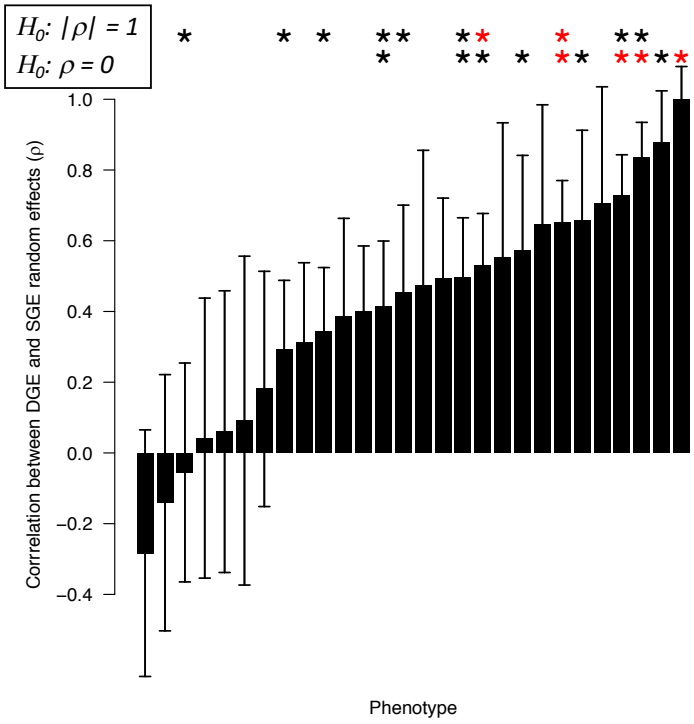


Figure 2 *Correlation coefficients ρ between SGE and DGE random effects (see Methods). Shown are correlation coefficients for 28 phenotypes with error bars denoting standard errors. The stars denote significance for rejecting H_0: ρ = 0* (i.e. SGE and DGE arise from different loci, bottom row) and *H_0: $|\rho|$ = 1 (i.e. pure phenotypic contagion, top row). Black stars (*) denote nominal P value < 0.05, red stars (*) denote Bonferroni-corrected P value < 0.05.*

sgeGWAS and dgeGWAS of 170 phenotypes

To map SGE, we calculated the "social genotype" of a mouse at a variant as the sum of the reference allele dosages across its cage mates at the variant[17,18], 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 random effects as in the model used for variance decomposition. In the sgeGWAS we also accounted for DGE of the tested variant, by including direct genotypes at the variant as a covariate (See Methods). Similarly, in the dgeGWAS we included social genotypes at the variant as a covariate. We hereafter refer to this strategy as "conditioning". We found that conditioning was necessary to avoid spurious associations in the sgeGWAS due to co-localised DGE (Supplementary Figure 2). As we show in the Supplementary Note, this problem originates from the use of each mouse as both focal individual and cage mate in the analysis, a strategy that has been used before to maximise sample size when all individuals are phenotyped and genotyped[17,18]. Importantly, spurious associations may arise even if all individuals are strictly unrelated (Supplementary Note). A power analysis suggested that conditioning may slightly decrease power to detect SGE in the absence of co-localised DGE, particularly when direct and social genotypes are highly correlated (Supplementary Figure 3a and 3c) but would increase power if the locus also gave rise to DGE (Supplementary Figures 3b and 3d).

In order to compare, for each phenotype, the results of sgeGWAS and dgeGWAS, we defined a locus as the average size of the 95% confidence interval in this population, namely 1.5Mb[19], and, following Nicod et al.[19], used a per-phenotype FDR approach to account for multiple testing (see Methods). sgeGWAS identified 24 genome-wide significant loci for 17 of the 170 phenotypes (FDR < 10%; Figure 3 and Supplementary Table 2). In comparison, dgeGWAS identified 120 genome-wide significant loci for 63 phenotypes at the same threshold (Figure 3 and Supplementary Table 3).

There was no overlap between genome-wide significant SGE and DGE loci *acting on the same phenotype*. However, variants at genome-wide significant SGE loci were enriched in small P values in the corresponding dgeGWAS (Supplementary Figure 4). Together these results suggest a partially distinct basis for SGE and DGE *acting on the same phenotype* (i.e. partially different loci and/or effect sizes), which is consistent with the results from the analysis of the correlation parameter ρ.

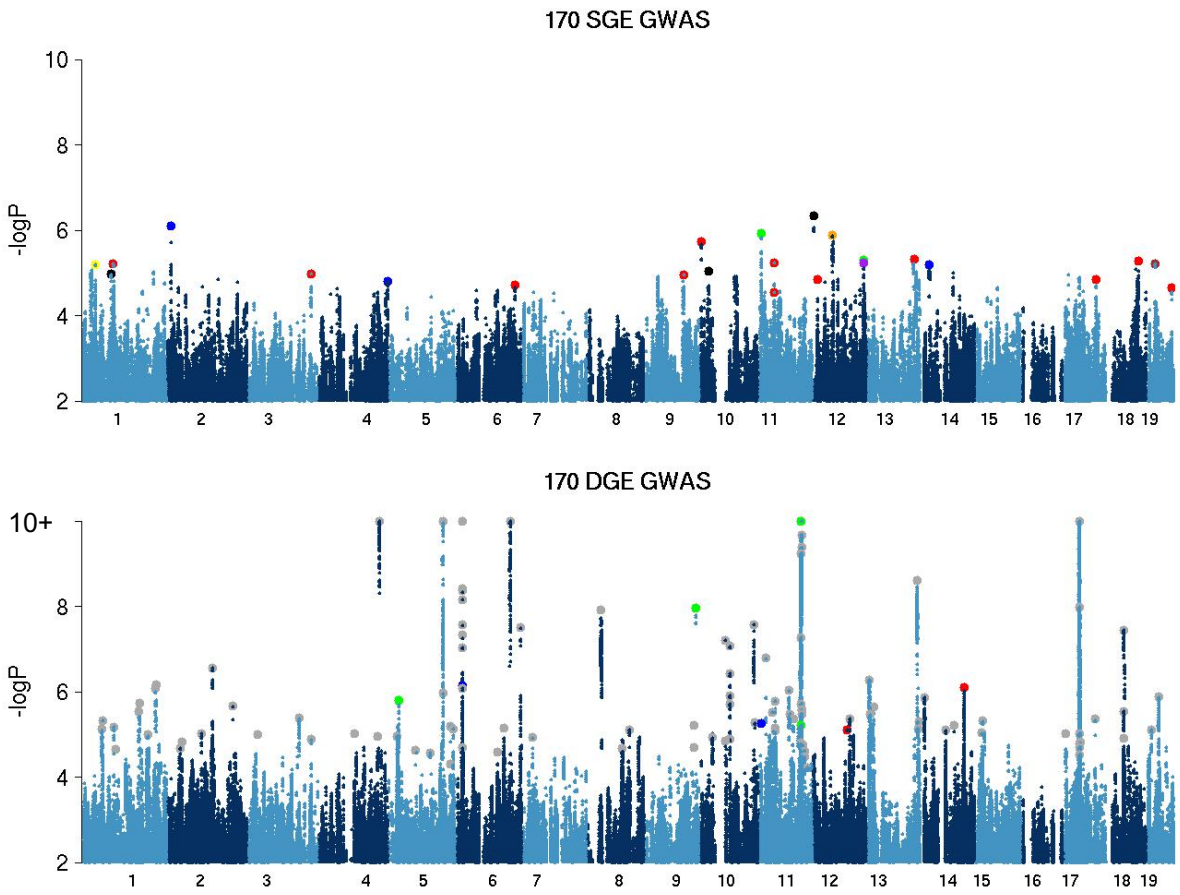
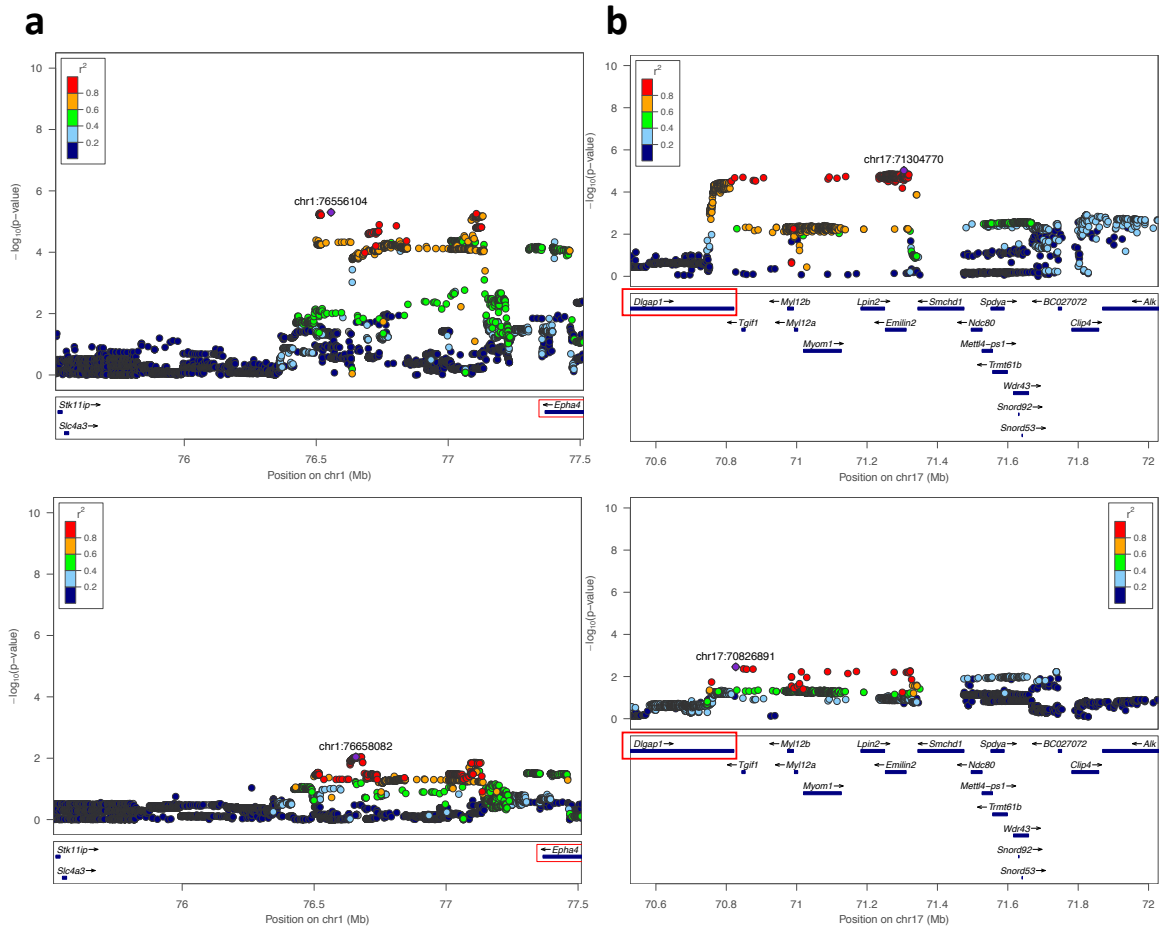


Figure 3 *Superimposed manhattan plots corresponding to to sgeGWAS (top panel) and dgeGWAS (bottom panel) for 170 phenotypes. DGE associations with a negative log P value greater than 10 were truncated at this threshold (as indicated by 10+); data points with negative log P values smaller than 2 are not shown. 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, includes 7 behavioural phenotypes with a detected SGE locus), adult neurogenesis (black, 2 phenotypes), immune (orange, 1 phenotype), haematological (yellow, 1 phenotype), blood biochemistry (blue, 2 phenotypes), bone phenotypes (green, 2 phenotypes), heart function (brown, 1 phenotype), and lung function (purple, 1 phenotype). 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 a genome-wide significant SGE association, the same color is used as in the SGE panel.*

Compared to many other mouse populations used for mapping, linkage disequilibrium decays rapidly in the CFW population, which facilitates identification of

candidate genes at associated loci[19,27]. At each genome-wide significant SGE locus we reran the association analysis using the full set of variants (rather than the pruned set used for GWAS) and prioritized candidate genes that were underneath or closest to the most significantly associated genomic segment, excluding certain categories of genes of unknown function (see Methods). This resulted in five genome-wide significant SGE loci with a single candidate gene (Supplementary Table 2).

An example is an SGE locus on chromosome 1 (Figure 4a, top panel) for immobility in the first two minutes of the Porsolt swim test, a measure of helplessness in CFW mice[28]. *Epha4*, the candidate gene at this locus, is critical for the development of the central nervous system (CNS) and synaptic plasticity in the adult brain[29]. *Epha4* mutant mice show a range of altered phenotypes including abnormal CNS morphology, altered limb coordination and lower locomotor activity, lower reproductivity, and poor maternal nurturing[30]. *Epha4* has also been associated with helplessness[31,32], but we found little evidence of DGE from *Epha4* on helplessness in our study (Figure 4a, bottom panel), in contrast with strong evidence of SGE. Expression data from the hippocampus of an independent sample of CFW mice[33], mined using Genenetwork[34] (see Methods), and a second genome-wide significant locus for helplessness in our study, located on chromosome 17, helped decipher the pathways mediating the social effects of *Epha4*: of the eight genes prioritized as candidate genes at the chromosome 17 locus (Figure 4b, top panel), one, *Dlgap1*, is among the ten genes whose expression in the hippocampus is most strongly correlated with that of *Epha4*. Thus, *Epha4* and *Dlgap1* of cage mates are likely involved in a common pathway affecting helplessness of focal mice. *Dlgap1* is a core component of the post-synaptic density and *Dlgap1* knock-out mice show selective deficits in sociability[35]. Hence, low sociability of cage mates most likely pathway explaining social effects of *Epha4* and *Dlgap1* on helplessness.



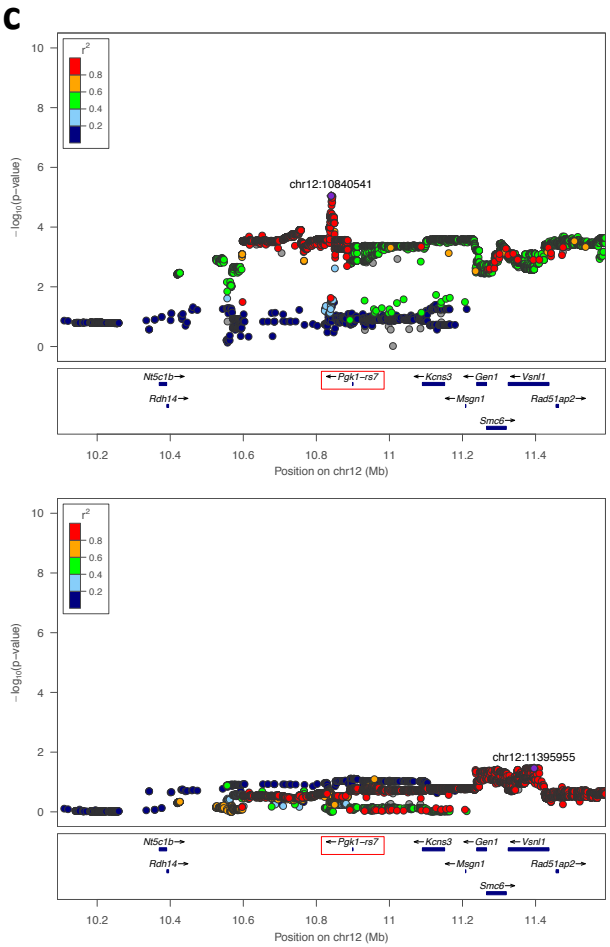


Figure 4 *Locus zoom plots for three genome-wide significant (FDR < 10%) SGE loci. In (a), (b), and (c) the top and bottom panels respectively show SGE and DGE of all variants at the locus. (a) Locus on chromosome 1 for immobility during the first two minutes of the Porsolt swim test, a measure of helplessness and model of depression in mice. Epha4 is the only candidate gene (see Methods) for SGE at this locus. (b) Locus on chromosome 17 for immobility during the last four minutes of the Porsolt swim test. Candidate genes for SGE include Dlgap1, whose expression is highly correlated with that of Epha4 in the hippocampus of CFW mice. (c) Locus on chromosome 12 for a measure of disrupted sleep, where Pgk1-rs7 is the only candidate gene for SGE.*

Among the other genome-wide significant SGE loci with a single candidate gene was a locus on chromosome 12 for a measure of disrupted sleep (Figure 4c, top panel). More precisely, after sharing a cage for 15 weeks mice were singly housed for three days so that their sleep could be recorded; the measure reported here was defined as the sum of differences in hourly sleep times between days 2 and 3[19]. Thus,

this measure captures unpredictable, disrupted sleep most likely resulting from mice struggling to adapt to their new housing conditions. The candidate gene at this locus, *Pgk1-rs7*, is a pseudogene of phosphoglycerate kinase 1 (*Pgk1*, located on chromosome X). Lack of linkage disequilibrium (see Methods) between variants in the vicinity of *Pgk1-rs7* and variants in the vicinity of *Pgk1* suggests our finding is not the result of sequencing read misalignment during genotyping. The perfect sequence match between the proteins encoded by *Pgk1-rs7* and *Pgk1,* the position of *Pgk1* on the X chromosome, and the existence of a functional pseudogene for the human phosphoglycerate mutase[36] (the enzyme acting on the product of PGK in glycolysis), together suggest that *Pgk1-rs7* might be a functional autosomal substitute for *Pgk1*, a process suggested to have enhanced male germline functions in evolution[37]. Neither *Pgk1-rs7* nor *Pgk1* have been associated with sleep and we found no evidence of DGE on sleep at this locus (Figure 4c, bottom panel), suggesting that other traits of cage mates are involved. Given that *Pgk1-rs7* is a marker of brown adipose tissue - the tissue responsible for non-shivering thermogenesis - and that its expression is activated by cold temperatures[38], our hypothesis regarding the pathways mediating social effects of *Pgk1-rs7* on sleep is as follows: first, *Pgk1-rs7* controls the heat produced by a mouse, which in turn determines the need for its cage mates to develop their own thermogenic capacity[39]. Indeed mice often huddle in the laboratory because ambient temperature (20° in the initial study) is well below their thermoneutral zone (30° [40]), and warm cage mates reduce the need for one mouse to produce its own heat[39]. Secondly, when the mice were separated for the sleep test after several weeks of sharing a cage, those with low thermogenic capacity likely got awaken by the cold conditions, resulting in disrupted sleep. Our results illustrate how the results of sgeGWAS may be used to generate hypotheses as to the traits of social partners mediating social effects.

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 loci than dgeGWAS (24 associations for 17 phenotypes and 120 associations for 63 phenotypes respectively). One possible reason for this is dilution of social genetic effects occurring when the focal mouse can only interact with one cage mate at a time.

We explored this possibility in a power analysis where allelic effects were fixed, three categories of minor allele frequencies (MAF) were considered (low, medium, or high; see Methods), and local DGE or SGE were simulated (see Methods). Briefly, we used two generative models for SGE : an additive model whereby SGE received by a focal mouse correspond to the sum of the genetic effects projected by its cage mates, and an average model, reflecting dilution, whereby SGE received by one mouse correspond to the average of the genetic effects projected by its cage mates. Our results show that power to detect SGE loci always increases with MAF and is higher than power to detect DGE when SGE were simulated under the additive model (Figure 5a). When SGE were simulated under the average model, however, power to detect SGE was lower than power to detect DGE. Thus, smaller effect sizes or dilution of social effects may have led to the detection of fewer genome-wide significant SGE associations compared to DGE associations.

Genome-wide significant SGE associations generally explained less phenotypic variance than genome-wide significant DGE associations: 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 and up to 40% (Figure 5b, Supplementary Table 2 and Supplementary Table 3); average values across all genome-wide significant associations were 1.8% for SGE and 2.7% for DGE.

Contrastingly, we found that genome-wide significant associations explained a similar proportion of the corresponding genetic variance for SGE and DGE (Figure 5c). More precisely, across 5 phenotypes with aggregate contribution of SGE greater than 5% and at least one genome-wide significant SGE association, we found that an average of 32.5% of the aggregate variance was explained by genome-wide significant associations. For DGE, that figure was calculated across 55 phenotypes and was equal to 32.1%. The proportion of aggregate variance explained by genome-wide significant associations may seem large given the relatively small number of genome-wide significant associations per phenotype (e.g. compared to humans[41]), but is consistent with studies of DGE in other outbred laboratory rodent populations[42,43] and are the result of a relatively small number of variants segregating in the CFW population and relatively high MAFs[19]. Our quantification of the proportion of genetic variance explained by genome-wide significant associations suggests SGE and DGE likely have similar levels of polygenicity. However, given the small number of

phenotypes where this fraction could be calculated, this conclusion will need to be re-evaluated when more SGE associations are discovered in other datasets.

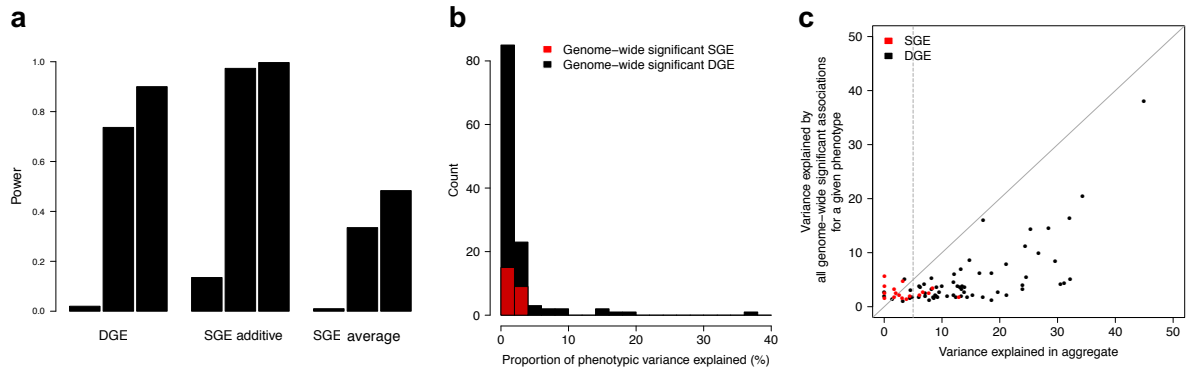


Figure 5 *Power to detect local SGE and DGE, and characterisation of the architecture of SGE and DGE. (a) Power to detect local genetic effects in simulations. Three types of local genetic effects were simulated: DGE, SGE arising from additive genetic effects across cage mates, and SGE arising from average genetic effects across cage mates (see main text and Methods). 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 based on direct genotypes). Power was calculated at a* genome-wide significance threshold of negative log P 5*. (b) Histogram of the proportion of phenotypic variance explained by individual genome-wide significant SGE (red) and DGE (black) associations (FDR < 10%). (c) Comparison, for each phenotype, of the variance explained by social (red) and direct (black) genetic effects in aggregate (x axis) and the total variance explained jointly by all genome-wide significant associations (y axis). Each dot corresponds to a phenotype with at least one genome-wide significant association. Only those phenotypes with aggregate SGE (or DGE) > 5% were considered to calculate the average values reported in the main text.*

Discussion

In this study we performed the comparative analysis of SGE and DGE acting on a broad range of phenotypes measured in outbred laboratory mice. For each phenotype we applied GWAS as well as variance decomposition techniques to identify individual loci giving rise to SGE in the CFW mouse population and compare the architecture of SGE to that of DGE. Our results demonstrate that sgeGWAS may be used to identify

novel genetic loci that contribute to inter-individual differences, illustrate how such loci can help infer the traits of social partners that affect the phenotype of interest, and suggest comparatively larger sample sizes will be necessary required for sgeGWAS compared to dgeGWAS.

For this study we chose to investigate not only phenotypes expected to be affected by social partners, such as behaviours and immune traits, but also phenotypes that were not *a priori* known or suspected to be socially affected, such as lung response to hypoxia, and muscle and bones characteristics. Indeed, our previous work, based on two different populations of mice[9], had suggested that SGE could affect a broad range of behavioural, physiological and morphological phenotypes. In this study we found that serum LDL cholesterol and alpha-amylase (a marker of pancreatic function), blood platelets, acute hypoxic response and healing from an ear punch, in addition to a number of behavioural and immune phenotypes, were significantly affected by SGE in aggregate (FDR < 10%, Supplementary Table 1). Thus, our study identified several phenotypes not previously known to be affected by the social environment and confirmed that SGE can affect a broad range of phenotypes. While the exact phenotypes affected by SGE in different populations vary, presumably due to differences in the pool of genetic variants segregating in the populations and other experimental variables[9], healing from an ear punch shows remarkable consistency across populations: it is indeed significantly and strongly affected by SGE, in aggregate, in all three mouse populations we have studied to date[9]. Unfortunately, there were no genome-wide significant SGE loci for this phenotype in this study, which prevented identification of the underlying mechanisms.

Our analysis of a broad range of phenotypes, together with testing of genome-wide variants, was also meant to provide a view of the architecture of SGE that is as unbiased as possible. We identified far fewer genome-wide significant SGE loci compared to DGE loci (24 and 120 respectively, Figure 3) and found that SGE loci tended to explain less phenotypic variation than DGE loci for the same phenotype (Figure 5b). Our results contrast with findings from two early mapping studies of SGE, which identified more SGE loci and found greater effect sizes for SGE loci as compared to DGE loci[15,17]. This discrepancy could be due to differences in the phenotypes studied, but more likely arises from the fact that one of those studies tested loci with genome-wide significant DGE only and the other one failed to account for DGE of the tested variant, thus not controlling the type 1 error rate if direct and

social genotypes were correlated (as we show in Supplementary Figure 2). Notably, our results are consistent with two other studies that mapped genome-wide SGE and DGE acting on a small set of traits: one that focused on mouse pup behaviour and found similar effect sizes for SGE and DGE loci[8], and one that focused on pecking behaviour in hens and identified DGE loci but no SGE loci[18]. Given the breadth of phenotypes considered in our study, lower effect sizes is likely to be a general feature of SGE loci, which implies future sgeGWAS will require larger sample sizes than dgeGWAS to be equally powered.

Given the effort that we predict will be necessary to carry out well-powered sgeGWAS, one goal of our study was to empirically evaluate the promise of sgeGWAS to identify genetic loci not identified by dgeGWAS and provide clues on yet unknown traits of social partners affecting the phenotype of interest. To do so, we calculated the correlation ρ between SGE and DGE acting on the same phenotype and tested whether it was different from |1|. We found that for 10 out of 28 phenotypes ρ was statistically different from |1| (nominal $P < 0.05$, Figure 2 and Supplementary Table 1), suggesting that different loci and/or different effect sizes give rise to SGE and DGE. Consistent with this observation, genome-wide significant sgeGWAS and dgeGWAS loci never overlapped. SGE and DGE loci were largely different even for phenotypes with prior evidence of "contagion", such as helplessness[21,44], a murine model for depression. Hence, our results motivate the use of sgeGWAS to discover more traits of social partners that influence depression and other phenotypes.

We focused on two genome-wide significant loci with a single candidate gene and no evidence of DGE to illustrate how sgeGWAS may help identify novel traits of cage mates affecting the phenotype of interest. A locus for helplessness on chromosome 1 featured *Epha4* as single candidate gene (Figure 4a), and we found that *Epha4* expression in the hippocampus of CFW mice was highly correlated with expression of *Dlgap1*, a candidate gene at another genome-wide significant SGE locus for helplessness in our study (Figure 4b). *Epha4* and *Dlgap1* both point to neurobehavioral traits of cage mates affecting helplessness of focal mice, and a report of selective deficits in sociability of *Dlgap1* knock-out mice[35] further indicates sociability of cage mates likely contributes to social effects on helplessness. Consistent with this hypothesis, single housing decreases the effectiveness of antidepressants in Swiss mice[45], which are genetically similar to CFW mice, and social

C57BL/6NHsd show increased anhedonia (another model of depression) when housed with less social BALB/cOlaHsd cage mates[46]. The other locus we investigated suggested an association between *Pgk1-rs7*, a gene associated with thermogenesis[38], and sleep disruption in cage mates (Figure 4c). We hypothesized that *Pgk1-rs7* controls the heat produced by a mouse, which in turn determines the need for its cage mates to develop their own thermogenic capacity[39,40] given that mice huddle to stay warm when sleeping; when the mice were singly housed for the sleep test, those with low thermogenic capacity got awaken by the cold conditions, resulting in the observed disrupted sleep. Consistent with this hypothesis, previous studies have described how increased cage density reduces thermogenic capacity even at ambient temperature very close to thermoneutrality (28° in Himms-Hagen et al.[47]) and how sleep quality is impacted by ambient temperatures[48].

One limitation of using SGE to study social effects is that it is blind to traits of social partners that are mostly non-genetically determined. Additionally, identifying traits of partners mediating social effect from SGE associations will likely be challenging, for the same reasons as it is challenging to infer “within-body” pathways of phenotypic variation from dgeGWAS results[13,49]. Additional data such as expression data collected in the cage mates (where SGE originate) may help improve the prioritization of candidate genes at SGE loci and identify the most relevant tissues.

Finally, our study makes several important methodological contributions that will help perform sgeGWAS, particularly in outbred populations where both DGE and SGE contribute to phenotypic variation. Specifically, we described the determinants of power for SGE and highlighted how dilution can reduce power to detect SGE (Figure 5a). We also showed how using the same individuals as both focal individuals and cage mates in the analysis leads to correlations between direct and social genotypes at a locus, which need to be accounted for to avoid spurious associations (Supplementary Note and Supplementary Figure 2). Similar correlations between direct and social genotypes, but potentially much stronger, 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[50,51], homophily between friends[10]). Our analysis approach is robust to spurious associations that arise in such situations.

Our study vastly improves our understanding of the architecture of SGE and will enable future sgeGWAS by guiding their design, analysis, and interpretation.

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/. Phenotypes were normalized using the boxcox function (MASS package[52]) in R; phenotypes that could not be normalised satisfactorily (transformation parameter lambda outside of -2 to 2 interval) were excluded. 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 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 4. 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 a few days 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 LD-pruned dosage matrix after standardizing the dosages for each variant to mean 0 and variance 1.

We excluded whole cages of mice 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 145 cages would have resulted in excluding 435 mice out of a total of 1,812, which would have led to substantially reduced power for sgeGWAS and dgeGWAS. Thus, we made a compromise and only excluded the 19 cages that were involved in 4 or more close pairs (57 mice excluded).

Variance decomposition

The same method as described in details in Baud et al.[9] was used. Briefly, the model used was:

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

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:

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

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 $P = I - \frac{11'}{n}$ [53,54].

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.

555 The Q value for the aggregate contribution of SGE was calculated for each phenotype
556 using the R package qvalue[55]. Significant SGE contributions were reported at FDR <
557 10% (corresponding to Q value < 0.1).
558
559 *Correlation between DGE and SGE*
560
561 The correlation ρ between $\underline{a_D}$ and $\underline{a_S}$ was calculated as:
562 $$\rho = \frac{\sigma_{A_{DS}}}{\sigma_{A_D} \times \sigma_{A_S}}$$
563
564 with ρ reflecting the correlation between SGE and DGE *acting on the same phenotype,*
565 similarly to how "traditional" genetic correlations measure the correlation between
566 DGE on two traits. The parameter ρ can actually be interpreted as the correlation
567 between DGE on the traits of cage mates mediating social effects and DGE on the
568 phenotype of interest itself.
569 We tested whether ρ was significantly different from 0 and whether |ρ| was significantly
570 different from 1 using a one-degree of freedom LLR test.
571
572 *Simulations for Supplementary Figure 1.*
573
574 Phenotypes were simulated based on the genotypes and cage relationships of the full
575 set of 1,812 mice. Phenotypes were drawn from model (0) with the following variances:
576 $\sigma^2_{A_D} = 15$, $\sigma^2_{A_S} = 8$, $\rho_{A_{DS}} = 0.47$, $\sigma^2_{E_D} = 22$, $\sigma^2_{E_S} = 16$, $\rho_{E_{DS}} = -0.97$, $\sigma^2_C = 26$. These variances
577 correspond to the median value of estimates across traits with aggregate SGE and
578 DGE > 5%. After building the phenotypic covariance matrix, the sample variance of
579 the simulations was calculated and used to calculate "realised" simulation parameters
580 from the "target" parameters above. The realised parameters were used for
581 comparison with the parameters estimated from the simulations.
582
583 *Definition of "social genotype" for sgeGWAS*
584

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. The same assumptions were made by Biscarini *et al.*[17] and Brinker *et al.*[18].

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[56].

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[57,58] with the covariance of the model estimated only once per phenotype, in the null 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, null) as "conditioning".

dgeGWAS was carried out by comparing model (2) above to the null model (3) 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, null) as "conditioning".

Identification of genome-wide significant associations

Because we wanted to compare the architecture of DGE and SGE *for each phenotype independently*, we adopted the per-phenotype FDR approach used by Nicod et al.[19].

In contrast to a study-wise FDR, this approach enables the direct comparison of paired SGEwas and DGEwas for the same traits.

The procedure we used to control the FDR accounts for the fact that we report loci rather than individual variants[59], where a locus is defined as the 1.5 Mb-wide window around a SNP (this window size is the average 95% confidence interval for DGE QTLs in [19]). More precisely, for each phenotype and for each type of genetic effect (social and direct), we performed 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). See [58,60] for references on this permutation approach. For a given P value x, the per-phenotype FDR can be calculated 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%.

Definition of candidate genes at associated loci

At each significantly associated locus we defined a 1.5Mb window centred on the lead variant, identified all the variants that segregate in this window based on the full set of 7M variants, and reran the sgeGWAS and dgeGWAS locally with all the variants at the locus. We highlighted those genes that are located within the most significantly associated segments and whose MGI symbol does not start by 'Gm', 'Rik', 'Mir', 'Fam', or 'Tmem' in order to focus on genes with known function and generate more tractable hypotheses on the pathways of social effects.

Calculation of linkage disequilibrium between variants in the vicinity of Pgk1-rs7 and variants in the vicinity of Pgk1

Dosages at LD-pruned variants in the regions chr12:10,091,326-11,591,326 and chrX: 105,500,000- 106,500,000 were used as input for PLINK2 to generate binary PLINK files, and linkage (R^2) was subsequently calculated between all pairs of variants.

Variance explained by a genome-wide significant association

The variance explained by a genome-wide significant SGE association was estimated in an extension of model (0) with additional fixed effects for both direct and social effects of lead SNPs at all genome-wide significant SGE loci (the lead SNP being the SNP with the most significant P value at the locus in the sgeGWAS). After fitting the model, the variance was calculated as:

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

where $sampleVar(C)$ is the sample variance of the covariance matrix in this model.

The variance explained by a genome-wide significant DGE association was estimated in a similar model but considering all genome-wide significant DGE associations and calculated as:

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

Variance explained jointly by all genome-wide significant SGE or DGE associations for a phenotype

The variance explained jointly by all significant SGE associations was estimated using the same model as above with all genome-wide significant SGE associations and calculated as:

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

The variance explained jointly by all significant DGE associations was estimated using the same model as above with all genome-wide significant DGE associations and calculated as:

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

Simulations for Supplementary Figure 2c and 2d.

Phenotypes were simulated based on the genotypes and cage relationships of the 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 random variants in the genome, and simulated variances of 0, 5, 20 or 50.

The results we show in Supplementary Figure 2c and 2d 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 for Supplementary Figure 3a-d, and Figure 3a.

Phenotypes were simulated based on the real genotypes but random cages. 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, σ^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). We simulated local SGE under two alternative generative models: an "additive" model by using Z as in model (2) (i.e. filled with 0s and 1s) or a "proportional" model by using $Z' = {}^{Z}/_{N}$. In all cases we simulated an allelic effect of 0.2 (similar to the average allelic effect estimated in the SGE GWAS). The sample variance of the simulated local DGE term is $var(Gb_D) = 2p(1-p)\,b_D^2$; it is $var(ZGb_S) = 2\mathrm{N}p(1-p)\,b_S^2$ for the local SGE term simulated under the additive

model, and $var\left(\frac{Z}{N}Gb_S\right) = 2\mathrm{N}p(1-p)/N^2\, b_S^2$ for the local SGE component simulated under the proportional model.

The results we show in Supplementary Figure 3a-d are based on simulations 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 no local DGE, 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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