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A complete absence of indirect genetic effects on brain gene expression in a highly social context

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Abstract

Genes not only control traits of their carrier organism (known as Direct Genetic Effects or DGEs) but also shape their carrier's physical environment and the phenotypes of their carrier's social partners (known as Indirect Genetic Effects or IGEs). Theoretical research has shown that the effects that genes exert on social partners can have profound consequences, potentially altering heritability and the direction of trait evolution. Complementary empirical research has shown that in various contexts (particularly in animal agriculture) IGEs can explain a large proportion of variation in specific traits. However, little is known about the general prevalence of IGEs. We conducted a reciprocal cross-fostering experiment with two genetic lineages of the clonal raider ant *Ooceraea biroi* to quantify the relative contribution of DGEs and IGEs to variation in brain gene expression (which underlies behavioral variation). We find that thousands of genes are differentially expressed by DGEs but not a single gene is differentially expressed by IGEs. This is surprising given the highly social context of ant colonies and given that individual behavior varies according to the genotypic composition of the social environment in *O. biroi*. Overall, these findings indicate that we have a lot to learn about how the magnitude of IGEs varies across species and contexts.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/mec.16686

Introduction

When individuals interact, the phenotype of one individual depends not only on its own genotype, but also on the genotypes of its social partners. Such between-individual genotype-phenotype interactions are known as Indirect Genetic Effects (IGEs)[1]. The presence of IGEs implies that the genotype-phenotype relationship is not one-to-one, as assumed in standard evolutionary models, but many-to-many, and the evolutionary consequences of this are profound. Theoretical work has shown that consideration of IGEs can substantially increase estimates of heritability and can alter the rate and direction of evolution[1–4]. The importance of IGEs has also been demonstrated empirically in studies on livestock, where these effects have been harnessed to improve breeding programs. IGEs account for 42% of feed intake and 27% of growth rate in domestic pigs[5], and an IGE-based breeding program in White Leghorns reduced annual mortality from 68% to 9% over four generations while increasing individual productivity[6]. Furthermore, IGEs are of potential medical relevance. They account for 29% of variation in health-related traits in mice, and influence human dietary traits and mental health[7,8].

IGEs are likely to be particularly relevant to behavioral phenotypes (and their underlying gene expression profiles) because behavior is inherently responsive to social context. Several studies using social model species have identified IGEs on behavior-related gene expression. Hundreds of genes were found to be differentially expressed in the brains of honeybees according to whether the social environment comprised bees of a 'high hygienic' or a 'low hygienic' genotype[9]. IGEs have also been found to strongly affect gene expression in the socially polymorphic system of the red imported fire ant, where some colonies have only one queen while others have multiple queens. Variation in queen number is controlled by a supergene, and workers either cull or tolerate supernumerary queens according to the frequencies of different supergene haplotypes in the colony[10]. Whole-body worker gene expression profiles were found to associate more strongly with the genotypic composition of the colony than with the genotype of the individual at the supergene[11].

While the important contribution of IGEs to behaviorally relevant gene expression has been shown in several specific contexts, little is known about the prevalence of these effects across different species or social contexts. Here we quantify how much of variation in brain gene expression can be explained by IGEs from adult nestmates versus direct genetic effects (DGEs) in the clonal raider ant *Ooceraea biroi*. We use *O. biroi* as a model because of its unusual reproductive system. *O. biroi* has lost its queen caste, and instead, unmated workers reproduce via parthenogenesis. Consequently, the species comprises multiple distinct genetic lineages with lineage-specific behavioral patterns (e.g., in degree of task specialization and level of extranidal activity)[12–16]. Using two of these lineages ('B'

and 'M'[14]) we performed a reciprocal cross-fostering experiment, transplanting newly-eclosed larvae so that the fostered individuals experienced their adoptive social environment during development and as adults. We performed RNA-seq on individual brains of fostered individuals (variation in brain gene expression is most relevant to behavioral variation) and show that thousands of genes are differentially expressed according to the genotype of the focal individual. Surprisingly, however, not a single gene was differentially expressed according to the genotype of the individuals constituting the social environment. This result suggests that the magnitude of IGEs may vary considerably between species and social context, and that IGEs may be negligible even in highly social contexts.

Methods

Lab stock colonies of lineages M and B were synchronized such that eggs hatched into larvae on the same day across colonies (reproduction is cyclic in *O. biroi* colonies, with all ants tending to lay eggs on the same day, and with eggs hatching into larvae on the same day[17,18]). On the day of larval hatching, larvae were collected and placed in groups of nine into Petri-dishes that each contained nine ants of either lineage B or lineage M. Small groups occur naturally in this species (field-collected colonies generally contain a dozen to a few hundred individuals[14,17,18], have high fitness, and exhibit complex social behaviour[19,20]). The fostering of newly-hatched larvae ensured that individuals experienced their adoptive social environment throughout larval and pupal development. We monitored the experimental sub-colonies daily (at 9am) to check for adult eclosion. The first of the fostered individuals to eclose as an adult was sampled on the morning of eclosion. These samples ('day 1') were used to investigate how much variation in brain gene expression was determined by direct and indirect genetic effects during development. When the second individual eclosed as an adult, the rest of the fostered brood were removed. This removal allowed for the focal adult to experience a genetically homogeneous social environment. The sub-colonies were then maintained at 25°C, 60% RH, and under 12 h:12 h light:dark cycle. The focal adult was sampled after eight days ('day 8'), which is long enough to allow interaction with social partners but not so long that some of the sub-colonies would switch to the reproductive phase and introduce another source of variation. The eight-day-old individuals were used to investigate the direct and indirect genetic effects on brain gene expression from across development and adult life.

We initially established 20 experimental sub-colonies per condition. Several sub-colonies failed, and final sample sizes are reported in Table 1.

Table 1: Sample sizes for the four conditions at two time-points

	Focal of genotype B	**Focal of genotype M**
Environment of genotype B	Day_1 = 7	Day_1 = 14
	Day_8 = 10	Day_8 = 12
Environment of genotype M	Day_1 = 9	Day_1 = 20
	Day_8 = 10	Day_8 = 14

RNA extraction, library preparation, and sequencing Upon collection, ants were anaesthetized on ice and brains were dissected out in 1x PBS, and homogenized in 1ml of TRIzol reagent with ceramic beads. Homogenized samples were incubated for 5 min at room temperature (RT). Chloroform (200µl) was added, samples were vortexed, and then incubated for 5 min at RT. Samples were centrifuged (25s at 12,000 rpm and 4°C) and the upper aqueous layer (~ 500µl) was transferred to a new tube with Isopropanol (650µl) and Glycogen blue (1 µl; RNAse-free, Invitrogen, 15mg/ml, #AM9516). Samples were vortexed and incubated overnight at -20°C. Samples were then centrifuged (30 s at full speed at 4 °C), the supernatant was discarded, and EtOH (1ml at 80%) was added. Samples were then vortexed and centrifuged (5 min at 12,000 rpm at 4°C). The supernatant was discarded and EtOH (1ml at 70%) was added. Samples were then vortexed and centrifuged again (5 min at 12,000 rpm at 4°C). The supernatant was removed and the pellet was allowed to dry (15 - 20 s) at RT. The pellets were resuspended in nuclease-free water. The extractions were performed in six batches. To prevent batch effects from confounding IGEs or DGEs, the four conditions for the day 1 samples were extracted in different batches, and the day 8 samples were approximately blocked across two batches.

The KAPA Stranded mRNASeq Library Preparation Kit (#KK8421) was used for library preparation. Samples were sequenced (single-end) across seven lanes of an Illumina HiSeq 2500, blocking samples of different conditions across the lanes.

Gene expression analysis Reads were mapped using the *O. biroi* genome (GCF_003672135.1_Obir_v5.4) and annotation (GCF_003672135.1_Obir_v5.4_genomic.gtf) with STAR v. 2.7.8a[21]. First, genome indices were generated using --runMode genomeGenerate with --sjdbOverhang 100 and --genomeSAindexNbases 12. Mapping was then run with --twopassMode Basic. Mapped reads were counted with FeatureCounts, with -Q 10 (Subread v. 2.0.2[22]). After mapping and counting, we obtained 16.9±3.16 million reads per individual (mean±sd). Before running the differential expression analysis, we filtered out genes with an average number of reads <10 across all samples (this removed 4,353 out of 13,755 genes). We used DESeq2 (which is based on

a negative binomial generalized linear model) to identify genes that were differentially expressed by time-point (model: age). We then looked within time-point to identify genes that were differentially expressed according to (i) direct genetic effects, while controlling for indirect genetic effects (model: genotype of social environment + genotype of focal), and (ii) indirect genetic effects, while controlling for direct genetic effects (model: genotype of focal + genotype of social environment). We additionally considered all samples together to investigate how many genes are differentially expressed by (i) direct genetic effects when controlling for indirect genetic effects and age, and (ii) indirect genetic effects when controlling for direct genetic effects and age. We re-ran these models adding terms for technical batch effects (sequencing lanes and extraction groups), and then again to test for in an inter- action between direct and indirect genetic effects (model: age + lane + extraction group + genotype of focal + genotype of social environment + genotype of focal:genotype of social environment).

In DESeq2, significant differential expression is assessed with a Wald test, and the Benjamini-Hochberg procedure is used to obtain multiple-testing adjusted p-values. Genes are considered as significantly differentially expressed if the adjusted p-value is < 0.1, which is default in DESeq2.

Results and Discussion

There were substantial differences in the brain gene expression profiles of 1- and 8-day-old ants. The two time-points clearly separated along the first principal component of a PCA, with 7,370 genes (78% of the genes that passed the low-counts filter) being significantly differentially expressed (3,645 genes were up-regulated at day 8 and 3,725 genes were up-regulated at day 1; Fig. 1). There was clearly more scatter at the earlier time-point. To quantify this, we calculated the standard deviation in expression for each gene in the two age groups separately and found that there was ~37% more variation in the brain gene expression profiles of the 1-day-old than in the 8-day-old ants (mean difference in standard deviation of normalized expression per gene between ants of the two time-points = 91 copies; Wilcoxon rank-signed test p-value < 0.001). This difference is likely because brains undergo rapid changes in gene expression during and immediately after eclosion[23,24], and so the 24 hours of variance in age within each time-point introduced larger transcriptomic variation among the 1-day-old than among the 8-day-old ants.

At both time-points, many genes were differentially expressed according to the genotype of the focal individual (i.e., DGE). In 1-day-old ants, 1,966 genes (20.9%) were significantly differentially expressed between the brains of lineage M and lineage B ants (while controlling for social environment), and in 8-day-old ants 1,268 genes (13.5%) were differentially expressed (Fig. 2A,C). At both time-points approximately half of the significantly differently expressed genes were up-regulated in

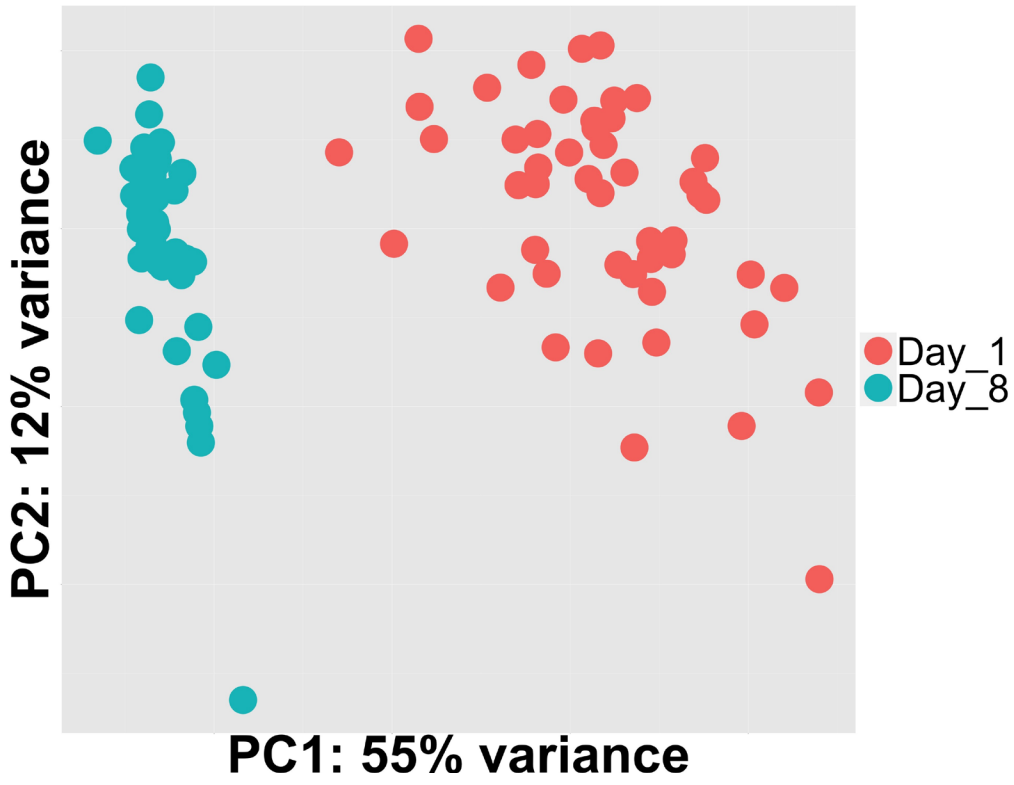

Figure 1: **Age had a considerable effect on brain gene expression.** There were clear differences in the brain gene expression profiles of adults on the first day of emergence, and eight days post-emergence. Color indicates age.

individuals of each genotype (Fig. 2B,D). In striking contrast, not a single gene was differentially expressed according to the genotypic composition of the social environment (i.e., IGE) when controlling for the genotype of the focal individual at either time-point (Fig. 2A,C). Similarly, when considering data from both time-points together, 1,622 genes (17.3%) were differentially expressed by DGEs while controlling for age and IGEs, while 0 genes were differentially expressed by IGEs when controlling for age and DGEs. When additionally controlling for extraction batch and sequencing lane 770 genes (8.2%) were differentially expressed by DGEs and 0 genes were differentially expressed by IGEs (both of these technical batch effects were small: seven genes were differentially expressed by extraction group, and 42 by sequencing lane when controlling for age, genotype of focal individual, and genotype of the social environment). These findings suggest that indirect genetic effects do not explain variation in brain gene expression in *O. biroi*. We cannot rule out that with a larger sample size we would have detected genes differentially expressed by IGEs. However, given the number of genes differentially expressed by DGEs, we can estimate that DGEs have at minimum a 1000-fold greater impact on brain transcriptomic variation than IGEs from adult ants under our experimental conditions.

This finding is surprising for two reasons. First, there is evidence for IGEs on adult behavior in

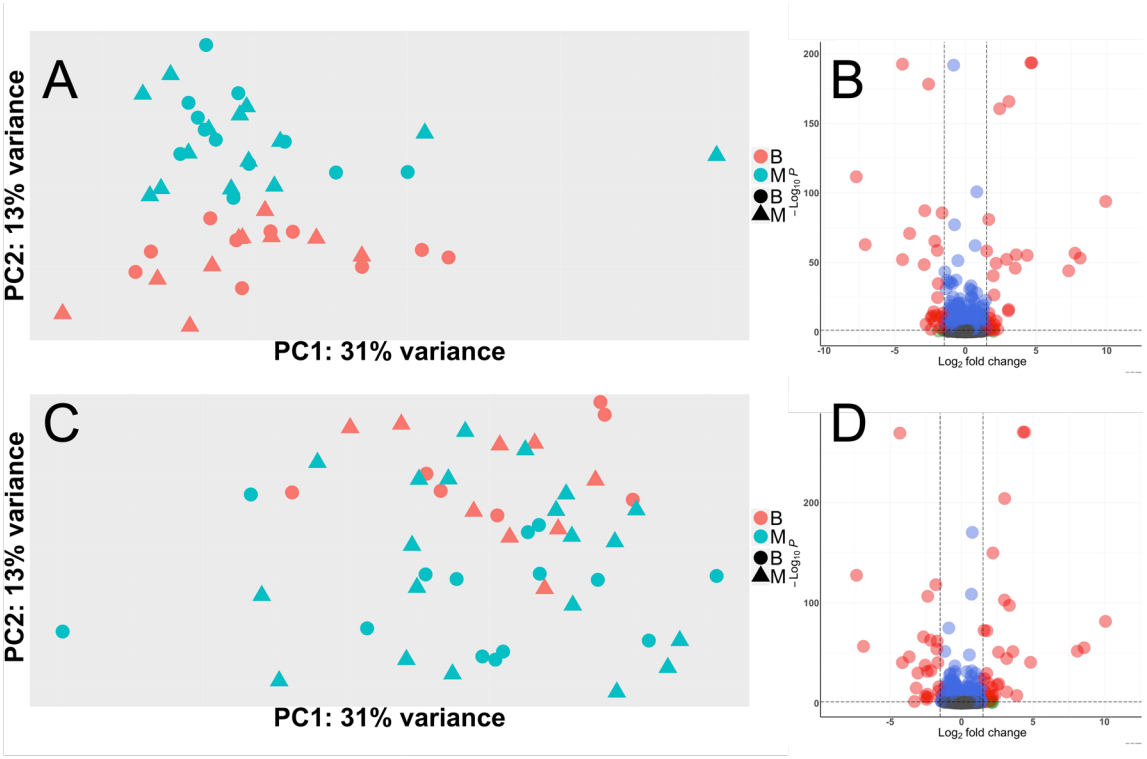

Figure 2: **Brain gene expression depended on direct and not indirect genetic effects.** In both PCAs color indicates individual genotype, and shape indicates the genotype of the individuals constituting the social environment. (A) Brains of 8-day-old ants, where there is clear clustering according to the genotype of the focal individual. (B) Volcano plot of genes differentially expressed according to the genotype of the focal individual among 8-day-old ants. (C) Brains of 1-day-old ants, where there is weak clustering according to the genotype of the focal individual. (D) Volcano plot of genes differentially expressed according to the genotype of the focal individual among the 1-day-old ants.

O. biroi. The proportion of time that an individual of a given genotype spends away from the nest (a proxy for foraging) varies according to the genotypic composition of the social environment[15,16,25]. We additionally observed IGEs on the duration of larval development time, showing that the behavioral differences between workers of different genotypes are relevant for larval development. Larvae raised by B workers took significantly longer to develop than larvae raised by M workers, which is consistent with B workers spending less time foraging (lm: larval development duration ~genotype of larvae + genotype of workers. Larval genotype p-value = 0.295, adult genotype p-value 0.00709; Fig. S1)[16]. Several recent studies have reported a non-correspondence between behavioral patterns and patterns of brain gene expression. In the red imported fire ant, adult behavior varies according to the genotypes of individuals present in the social environment and yet cross-fostering young adults showed no significant effect of the social environment on brain gene expression[26]. In the paper wasp *Polistes dominula*, all workers exhibit substantial changes in brain gene expression if the queen is removed from the colony but only few individuals transition to exhibit queen-like phenotypes[27]. One possible explanation for these apparent inconsistencies

between behavioral patterns and brain gene expression profiles is that general behavioral heuristics (e.g., leave the nest to collect food when larval demand passes a given threshold, or develop into a queen if others appear relatively smaller or weaker) are associated with broad-scale gene expression patterns in the brain, but that the actual performance of a specific behavior is associated with comparatively subtle changes in brain gene expression which are not readily detectable with a bulk-sequencing approach.

Second, the focal individuals were fostered on the day that they eclosed from eggs, and so experienced the adoptive social environment across larval and pupal development. In *O. biroi*, as in other social insects, the brood is completely dependent on the social care provided by adults. Moreover, in social insects, differences in provisioning regimes can have profound consequences on gene expression[28] and the developmental fate of females (queens and workers). In *O. biroi*, ants of different genotypes have been found to interact differently with brood (dedicating different proportions of their time to nursing), meaning that IGEs would be expected to influence development, and therefore brain gene expression upon eclosion[15,25,28].

In general, IGEs should increase in magnitude with group size[2,8]. However, this is unlikely to have a significant effect in *O. biroi*. Since these ants are not capable of individual recognition, what matters is the proportion of time spent in contact with individuals of given genotypes. In the nest, the workers are in constant contact - clumped together at maximal density - and so increases in group size should have only a marginal effect on the proportion of time individuals spend in contact with others of particular genotypes.

In conclusion, our study reveals an absence of IGEs in a context where they could be expected. This apparent absence suggests that IGEs may play a more minor role in determining patterns of brain gene expression and consequent behavior and can be more system-specific than previous evidence would suggest.

Acknowledgements

This study was funded by the European Research Council (ERC Advanced Grant, "resiliANT," no. 741491) and the Swiss NSF.

Data and materials availability

Raw RNA-sequencing data have been deposited in NCBI's Gene Expression Omnibus under accession number GSE210246. Metadata and code are available on GitHub at https://github.com/MO- Katy/IGEvDGE_ClonalRaiderAnt.

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