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Indirect genetic effects for social network structure in *Drosophila melanogaster*

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The position an individual holds in a social network is dependent on both its direct and indirect social interactions. Because social network position is dependent on the actions and interactions of conspecifics, it is likely that the genotypic composition of individuals within a social group impacts individuals' network positions. However, we know very little about whether social network positions have a genetic basis, and even less about how the genotypic makeup of a social group impacts network positions and structure. With ample evidence indicating that network positions influence various fitness metrics, studying how direct and indirect genetic effects shape network positions is crucial for furthering our understanding of how the social environment can respond to selection and evolve. Using replicate genotypes of *Drosophila melanogaster* fruit flies, we created social groups that varied in their genotypic makeup. Social groups were videoed, and networks were generated using motion-tracking software. We found that both an individual's own genotype and the genotypes of conspecifics in its social group affect its position within a social network. These findings provide an early example of how indirect genetic effects and social network theory can be linked, and shed new light on how quantitative genetic variation shapes the structure of social groups.

This article is part of a discussion meeting issue 'Collective behaviour through time'.

1. Introduction

The social interactions that individuals engage in, and the emergent structure of the social groups they are a part of, can shape individuals' entire lives in important ways [1–3]. One of the many factors contributing to social interactions and emergent social group structure is the genotypes of individuals comprising a group [3–6]. From an evolutionary perspective, the quantitative genetics of social interactions are particularly interesting: when the genotype of one individual affects the phenotype of an interacting partner—a phenomenon termed indirect genetic effects (IGEs)—this genetic component of the social environment indicates that the social environment itself can respond to selective pressures and evolve [7–9].

Theoretical and empirical work incorporating IGEs has seeded a better understanding of variation in social interactions and social dynamics [10–14]. However, models of IGEs have struggled to move beyond dyadic social interactions, to better mimic the common natural situation in which many individuals (and their genotypes and phenotypes) interact simultaneously [4,5,14–17]. This limitation is problematic for applying IGE theory to data from wild study systems, because individuals frequently engage in social interactions with multiple conspecifics, either simultaneously or sequentially. One of the ways IGE theory has been extended to apply to social groups is to take the average phenotype of a focal individual's social groupmates [5]. An issue that arises with this approach is that how individuals affect others is often not an average. Previous studies have demonstrated, for example, that some individuals can have a disproportionate influence on their social groups [18] and networks (e.g. [19]). Furthermore, how interacting individuals affect others

can depend on the specific individuals or genotypes they are paired with [20–27]. If the effect of interacting individuals depends on both their own genotype and the genotype of the individuals they are interacting with, then these effects can be considered a type of genotype-by-environment interaction, where the 'environment' is the IGEs imposed by the social environment [23–26,28,29]. However, we still know very little about how variability in IGEs and genotype-by-IGE interactions (also termed genotype-by-genotype epistasis) shape variation in group structure [30].

To move beyond studying IGEs in dyadic contexts, social network analysis provides a promising opportunity, as it allows us to measure how the effects of social group members cascade through networks of direct and indirect interactions [4–6,17]. Social network analysis has emerged as a widely used tool for describing how individuals' direct and indirect social interactions are nested within the emergent social structure of their group [1,2,31–34], but has only rarely been deployed in genetically informative samples that would allow estimation of IGEs [4,5,17].

Numerous studies have found that behavioural variation shapes the positions that individuals hold in their networks of social interactions (e.g. movement and exploratory behaviour [35,36]; aggression and policing behaviours [19,37,38]; group size preference and experience [6,29,39]). However, it remains unclear whether these behavioural effects on network structure have an underlying quantitative genetic basis, and if so, how genetic variation acts through direct genetic effects (DGEs) and IGEs to produce variation in network structure [4–6,40]. Extending our knowledge of how the genetic effects of social group members affect the structure and dynamics of networks of social interactions—i.e. IGEs within social networks—can allow us to better understand how variation in social structures forms, shapes behaviour and its genetic basis, and evolves [3–5,17].

Because the position that an individual holds in a network is inherently dependent on the direct and indirect social interactions occurring amongst all social group members, the effects of individuals' own genotypes on their own network position are difficult to parse from the IGEs of other social group partners [40]. For example, variation in the social behaviours of conspecifics in a social group could affect a focal individual's position in a network, even if the focal individual's social behaviours remain consistent. However, while an individual's network position may be relative compared to other individuals in its network, it is important to note that individuals' own behaviours can and often do shape their network positions [19,35,36,39,41–43]. So, an individual's social network position is expected to be an emergent property of its own behaviour, plasticity in its own behaviour across groups, and the behaviour and plasticity of groupmates.

As such, while the hypothesis that the genotypic composition of social groups should influence individuals' network positions seems intuitive, this is not a guarantee; and empirical evidence evaluating the effects of IGEs in social networks (or their absence) is lacking. Indeed, few studies have addressed how an individual's own genotype influences its position within a social network [37,44–46], and we know even less about how the genotypic composition of social groups and IGEs of interacting group members shape variation in network phenotypes [5,17,40]. A handful of studies have addressed how the genotypic makeup of a social group impacts various aspects of its structure and dynamics: social niche construction [28,47–49], exploratory behaviour [26], collective foraging [50], antipredator behaviour [10] and aggression [15,38]. Yet it remains unclear how the genotypes of multiple individuals within social groups impact the structure of their networks of social interactions. Merging studies of the quantitative genetic basis of social traits, IGEs, and social networks can open new windows into understanding the genetic basis and evolutionary potential of social group structures.

In this study, we started to bridge this gap in knowledge by examining how multiple individuals' genotypes and the genotypes of their social partners affected multiple measures of social network positions using replicate genotypes of *Drosophila melanogaster* fruit flies. We have previously demonstrated a heritable basis to social network position in this system [46]. Further, we found that social network position influenced measures of male and female fitness, underlining the importance of social network dynamics in this system. Here, we manipulated and varied the genotypic composition of entire social groups, allowing us to quantify the role of DGEs and IGEs in social network structure. We predicted that the genotypes present in a social group would influence the network positions of all members.

Specifically, we predicted that including genotypes known to differ in their eigenvector centrality (a measure of the 'importance' of an individual to the structure of its network based on its direct and indirect social connections [2,32,34]) in a different social setting [46] would also differ in their impact on their own social network positions, and those of their groupmates. An individual's eigenvector centrality can serve as a measure for how much that individual serves as a 'hub' of social interactions; i.e. an individual with high eigenvector centrality may engage in many social interactions and/or engages in social interactions with individuals who are themselves highly connected [2]. For example, female baboons who groom highly connected social partners thereby gain access to many more grooming partners, making them 'hubs' for this social behaviour (i.e. having high eigenvector centrality) [45,51]. We consider eigenvector centrality as a good 'test case' for understanding IGEs in social networks because eigenvector centrality: (i) is inherently an emergent property of many individual interactions and (ii) varies among genotypes and influences measures of fitness in our study system. In our study, the effect of the presence or absence of particular genotypes on a focal individuals' network positions would represent an IGE on the focal individuals' network positions. Further, we predicted that this IGE would be dose-dependent, such that the more individuals from the high- or low-eigenvector centrality genotype were present in the group, the stronger this effect would be. Finally, we tested whether the IGEs discovered, if any, were dependent on the identity of the other genotype present, representing a genotype-by-IGE interaction.

2. Methods

(a) Study system

Flies are a great study system to address questions about the quantitative genetic basis of social traits, as we can replicate the genotypes of individuals and entire social groups using multiple replicate genotypes [52]. Flies actively form social groups on food substrates in nature [53–55] and in the laboratory [48,56–58]. Additionally, flies have been shown to vary in social group preference [28,47]; to actively choose the social groups they are a

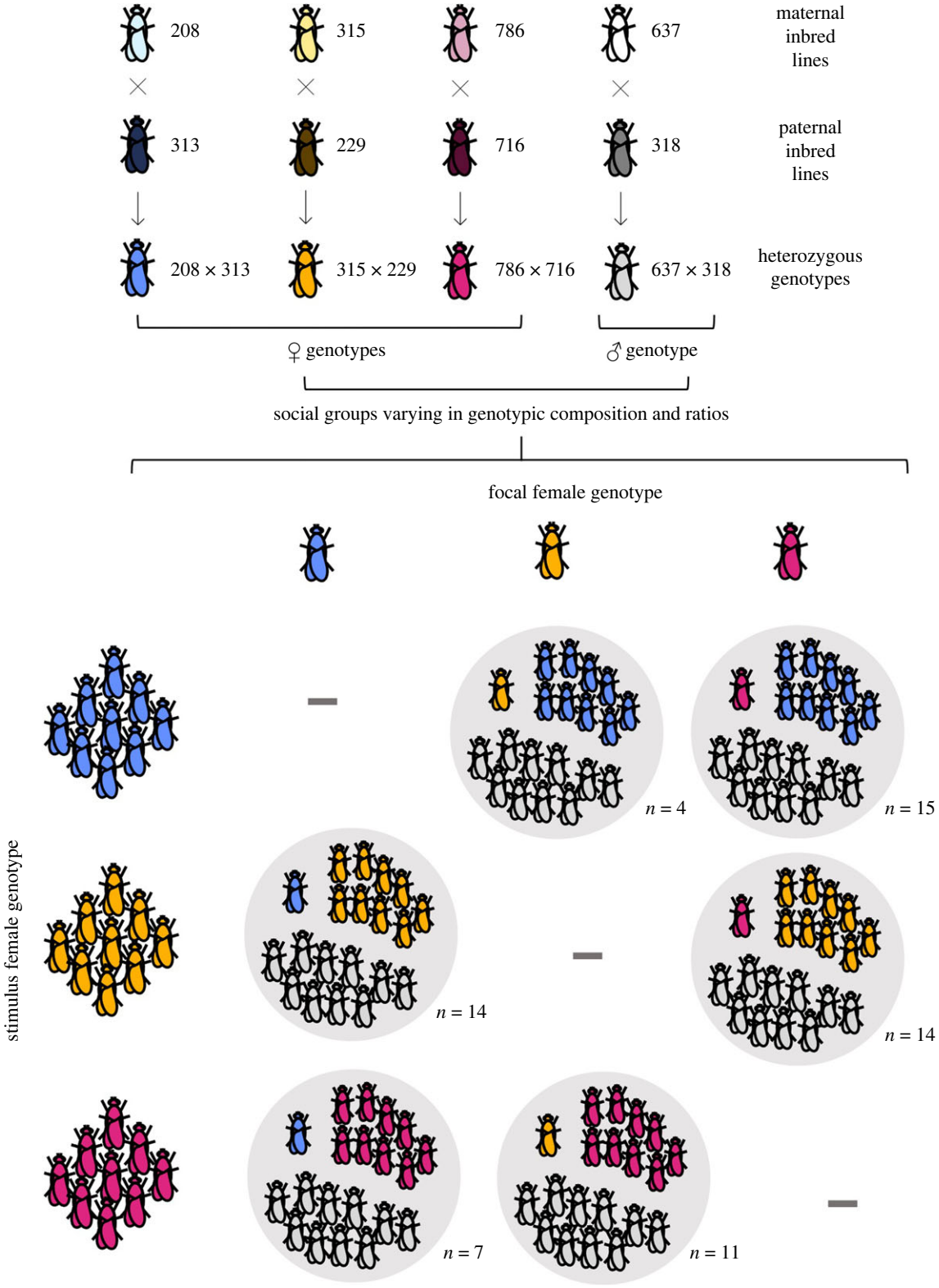

Figure 1. Overview of the experimental design indicating how heterozygous genotypes were derived from homozygous inbred lines of flies, and how social groups of variable composition and ratios of female genotypes were established. Genotype numbers refer to arbitrary indicators from the DGRP [52]. Sample sizes indicate the number of networks analysed for each social group treatment.

part of [48,49]; and to have non-random social networks of interactions [46,57,59].

(b) Genotypes and rearing

Heterozygous genotypes were created by performing mating crosses of inbred homozygous genotypes of *D. melanogaster* flies derived from the *Drosophila* genetic reference panel (DGRP), a collection of inbred lines derived from a natural population in North Carolina ([52]; figure 1). Each heterozygous genotype used in our experiment was generated by establishing a mating cross of 10 virgin females of homozygous lines 208, 315, 786 or 637 with 10 males of homozygous lines 313, 229, 716 or 318, respectively. Virgin females were collected from mating crosses 208 × 313, 315 × 229 and 786 × 716; and virgin males were collected from mating cross 637 × 318. Genotype numbers refer to arbitrary labelling within the DGRP and are not indicative of similarities or differences between genotypes [52].

Seeding the vials with a standard number of parents per vial (10 females and 10 males), allowed us to minimize variation in larval density. We chose to use heterozygous genotypes, as opposed to homozygous flies from inbred lines, to create individuals that are more representative of genetic variation found in the wild. Additionally, creating heterozygous genotypes allays concerns about potentially deleterious effects of homozygous recessive alleles in inbred homozygous lines [60,61].

We chose three heterozygous female genotypes to create social groups of variable genotypic compositions, because we have demonstrated that these genotypes vary in how critical they are to social network structure based on the strength of their direct and indirect social interactions (i.e. eigenvector centrality) in social groups of standardized genotypic composition [46]. We chose female genotypes that have been previously shown to vary in eigenvector centrality for three reasons. First, this measure of network position incorporates information about both an individual's direct and indirect social interactions, which is a key part of addressing our question of how an individual's own genotype, and the genotypes of its direct and indirect social partners, affects the network positions of a focal individual and its social groupmates. Second, eigenvector centrality is an inherently relative measure: if one or more individuals are highly central to group structure, there must be an individual(s) who is not as central to group structure, as not everyone within a group can have high eigenvector centrality. Third, an individual's eigenvector centrality in a group has been demonstrated to be affected by numerous phenotypes (e.g. age [62,63], sex and dominance rank [64] and exploratory behaviour [42]) with important consequences for numerous life history traits (e.g. mating frequency [46], fecundity [65], survival [65,66], food patch discovery [67], territoriality [68], predation [69] and disease [70,71]). We chose to manipulate both the genotypic identities and number of females of each genotype in the group, as our results from previous work indicate that female fruit flies receive more social interactions and are more central to the structure of their social groups than males [46].

All flies were reared, aged, and housed on a 12 : 12 light : dark cycle, at 24°C, 50% relative humidity, and on standard fly food (unless otherwise noted). Newly eclosed virgin adults were collected under light CO_2 anaesthesia. Each fly was marked with a unique paint colour on its mesothoracic segment, allowing us to visually distinguish individuals. Flies were then aged in same-sex groups for 3 days to allow for recovery from CO_2 anaesthesia and development to sexual maturation [72,73]. Females were housed in their specific treatment combinations (described below and in figure 1), and males with other males of the same genotype.

(c) Social groups

Groups were composed of 20 individuals (10 males and 10 females). In all groups, all 10 males were of a standardized heterozygous genotype (figure 1). The genotypic composition and genotypic ratio of females were varied. To vary the genotypic composition of groups, each social group contained two different heterozygous female genotypes out of the three total in our experiment (see §2b above), for a total of three possible genotypic compositions. Further, we manipulated the ratio of each genotype, where a given female genotype served as either the minority (one individual, referred to as the 'focal genotype') or the majority (nine individuals, referred to as the 'stimulus genotype') in each group (figure 1). This allowed us to address not only how the IGEs of an individual's social group partners affect its network position, but how the genotype of a single individual could affect the structure of its social groupmates. Every genotype was measured as the focal and stimulus individuals in each genotypic composition combination, representing a full-factorial design totalling six treatment genotypic composition and ratio combinations (figure 1).

(d) Fly behaviour and social network analysis

At the start of trials, males and females were released into a 10 cm petri dish layered with fly food (58.8 g nutritional yeast, 133.7 g malt sugar, 27 g agar, 11.1 ml tegocept acid mix (70 g tegocept/270 ml H_2O) and 3 ml propionic acid; per 1 l H_2O) to form a social group.

Social groups were video recorded during the first hour of light (when flies are most active) over the course of 2 days, beginning the morning after social groups were established [74]. Videos were recorded for 20 min using Nikon D3300 cameras at 30 fps and 10.22 ± 0.24 pixels mm^{-1}. The position and orientation of each fly in each video were quantified using the motion-tracking software Caltech FlyTracker 1.0.5 [75]. The fly tracks in each video were manually verified to ensure all tracking identities were accurate and consistent.

We measured the weighted and directed social interactions occurring between every pairwise combination of flies using the tracking output [76]. This allowed us to create a social interaction matrix for each video. Three criteria dictated whether or not two flies were socially interacting: (i) the distance between the two flies was less than 2.5 average fly body lengths, (ii) a social partner was within a 320° field-of-view and (iii) these first two criteria were maintained for a minimum duration of 0.6 s [77]. These interaction criteria have been previously demonstrated to filter out random social interactions, such as two flies walking by one another without socially engaging [77]. Each edge in our interaction matrices encompassed the total duration of time any two flies spent interacting during a video, given our three interaction criteria. Because all individuals were observed and present throughout the entirety of every video, this allowed us to directly proceed with network analyses without having to compute association indices to account for sampling errors or biases, or carry this estimation error forward in subsequent analyses [78–81]. Using the R package igraph 1.2.4.2 [82], we analysed four of the most commonly studied individual-level social network positions: instrength—the amount of time other individuals spend socially engaging with an individual; outstrength—the amount of time an individual spends socially engaging with other individuals; weighted and directed clustering coefficient—how interconnected an individual's direct social partners are to one another (i.e. cliquishness); and weighted and directed eigenvector centrality—how critical an individual is to the overall structure of the group based on the strength of its direct social connections, the strength of its partners' connections, the strength of its partners' partners' connections, etc. [2,32,34] (electronic supplementary material, figure S1).

(e) Replication

One hundred and twelve social groups were created, representing 18–19 replicates of each genotype ratio/composition: 1 : 9 ratio of genotypes 208 × 313 and 786 × 716, respectively ($n = 19$); 1 : 9 ratio of 208 × 313 and 315 × 229 ($n = 19$); 1 : 9 ratio of 786 × 716 and 315 × 229 ($n = 19$); 9 : 1 ratio of 208 × 313 and 786 × 716 ($n = 19$); 9 : 1 ratio of 208 × 313 and 315 × 229 ($n = 18$); 9 : 1 ratio of 786 × 716 and 315 × 229 ($n = 18$). Social groups were excluded from analyses if any flies died or escaped before they were videoed. This excluded over half of our social groups from analyses, as keeping all 20 flies in a group alive throughout the duration of the experiment was challenging. However, doing so was necessary, because variation introduced by the presence of a dead individual and subsequent changes in group size caused groups to no longer be replicated within each genotype ratio/composition treatment. For intact social groups, up to two videos were taken (one/day over the course of 2 days). Forty-four independent social groups had fully tracked videos, 21 of which were videoed on both days, resulting in 65 tracked videos of social groups used for network analysis. Thus, our

final dataset included 4–15 replicates per genotype ratio/composition treatment: 1 : 9 ratio of genotypes 208 × 313 and 786 × 716, respectively ($n = 7$); 1 : 9 ratio of 208 × 313 and 315 × 229 ($n = 14$); 1:9 ratio of 786 × 716 and 315 × 229 ($n = 14$); 9 : 1 ratio of 208 × 313 and 786 × 716 ($n = 15$); 9 : 1 ratio of 208 × 313 and 315 × 229 ($n = 4$); 9 : 1 ratio of 786 × 716 and 315 × 229 ($n = 11$) (electronic supplementary material, table S1).

(f) Analyses of direct and indirect genetic effects on network structure

Because all males had the same standardized genotype, we could not address how a male individual's genotype (DGE) affected their own network position. We could, however, estimate DGEs for female network positions, as female genotypes varied within and across social groups. As such, networks were constructed for the entire social group, but then data from females and males were analysed separately to ask different questions about the quantitative genetic causes of variation in social network position.

For females, we analysed how an individual's own genotype (DGE), the genotype of the female(s) in their group (IGE), and their genotypic frequency in their social group impacted their social network positions. We also analysed how all pairwise combinations of interaction effects between DGEs, IGEs, and genotypic frequency effects impacted females' network positions.

For males, we analysed how IGEs of the specific genotypic composition and ratio of females in their group impacted males' network positions. The IGE of the singular unique female genotype in each group is referred to as an IGE of the 'focal genotype', and the IGE of the nine female genotypes that were consistent within a social group is referred to as an IGE of the 'stimulus genotypes'. The presence of IGEs on males was tested based on the significance of stimulus and/or focal female genotype fixed effects. We also tested for an interaction effect between the focal and stimulus female genotypes that males were paired with on *males*' network positions.

In all models analysing the effects on network structure, the effects of DGEs, IGEs, an individual's genotypic frequency in the group, and interactions between these effects were treated as fixed factors. Genotypic effects (DGEs and IGEs) were treated as fixed factors because genotypes were chosen non-randomly based on prior information (see §2b), and because there were only three levels of genotype for females. All models also included a random effect of the identity of the individuals' social group, to account for the fact that each individual was a sub-sample of its social group.

(g) Model fitting

All analyses were conducted in R v.3.6.2 [83]. Network positions of instrength and outstrength were analysed using Poisson-distributed generalized linear mixed models (GLMMs), as these measures of network position are counts; and network positions of clustering coefficient and eigenvector centrality were analysed using linear mixed models (LMMs) using R package lme4 1.1 [84]. Model fits were assessed using the package DHARMa 0.2.7 [85]. Accommodations for overdispersion were applied as needed using an observation-level random effect [86].

(h) Inference

Fixed effects and all interactions were assessed using Type III Wald χ^2 tests, and non-significant interactions were removed [87].

Because measures of network position are non-independent within social groups, meaning one individual's network position inherently informs other individuals' network positions, we employed nodal permutation tests to test for the significance of DGEs, IGEs, genotypic frequency effects, and all pairwise interactions between these effects on females' network positions [88–90]. Since our network data were generated via automated tracking and manual verification of video data, and we could ensure that all individuals and social interactions between them were represented in our networks (i.e. no missing data); nodal permutations were employed as the most appropriate means of testing the significance of effects, while avoiding the possible risk of elevated error rates associated with pre-network or datastream permutations [90,91]. In each permutation test, 1000 null datasets were generated by randomizing the genotypic identities of females within each social group (DGEs), the identity of the other female genotype in the group (IGEs), and their genotypic frequency in a social group in tandem. Type III Wald χ^2 test statistics from the observed data were compared to Type III Wald χ^2 test statistics generated from the null datasets to ascertain the significance of effects. Additionally, we sought to estimate the variance 'explained' by significant DGEs and IGEs on females' network positions using the R package MuMin 1.43.17 [92]. Pseudo-R^2 values were obtained by estimating the variance explained by DGEs and IGEs on network positions using models with each fixed effect included individually, but otherwise constructed identically as described in §§2f,g.

For males, permutation tests were not required to inform the significance of IGEs of focal and stimulus female genotypes on males' network positions, because the genotypic identity of the focal and stimulus females was consistent within each social group. As such, there was nothing to permute, and significance was assessed using Type III Wald χ^2 tests on the observed male data.

3. Results

(a) Direct and indirect genetic effects on female network positions

Female genotypes significantly differed in three measures of network position. We found DGEs on how much time other individuals interacted with a given individual (instrength, $\chi^2 = 5.619$, p-value derived from nodal permutations, hereafter $p_R = 0.001$, pseudo-$R^2 = 0.051$), how much time individuals spent engaging with other individuals (outstrength, $\chi^2 = 3.202$, $p_R = 0.001$, pseudo-$R^2 = 0.044$), as well as how central individuals were to the structure of their social groups (eigenvector centrality, $\chi^2 = 13.095$, $p_R < 0.001$, pseudo-$R^2 = 0.035$; figure 2; table 1). We did not find evidence to suggest a DGE on how cliquish individuals were (clustering coefficient, $\chi^2 = 12.149$, $p_R = 0.503$; figure 2; table 1).

We found IGEs for eigenvector centrality: the genotypic identity of a female's groupmates significantly impacted how central she was to the structure of the network (eigenvector centrality, $\chi^2 = 3.270$, $p_R = 0.016$, pseudo-$R^2 = 0.020$). More specifically, when a female was paired with genotype 208 × 313 (estimated marginal mean (EMM) eigenvector centrality = 0.214), 315 × 229 (EMM eigenvector centrality = 0.214) or 786 × 716 (EMM eigenvector centrality = 0.241), her eigenvector centrality values had an EMM of 0.230, 0.215 and 0.223, respectively (figure 2*g*,*h*). Notably, when a female was paired with a genotype with one of the lowest eigenvector centrality values (208 × 313), she in turn was more likely to have the highest eigenvector centrality value compared to being paired with the other female genotypes. This matches our prediction of an IGE of being paired with a genotype of a given eigenvector centrality value having an inverse effect on one's own eigenvector centrality. We did not find evidence for an IGE on our other tested measures of network position (instrength, outstrength or clustering coefficient; table 1).

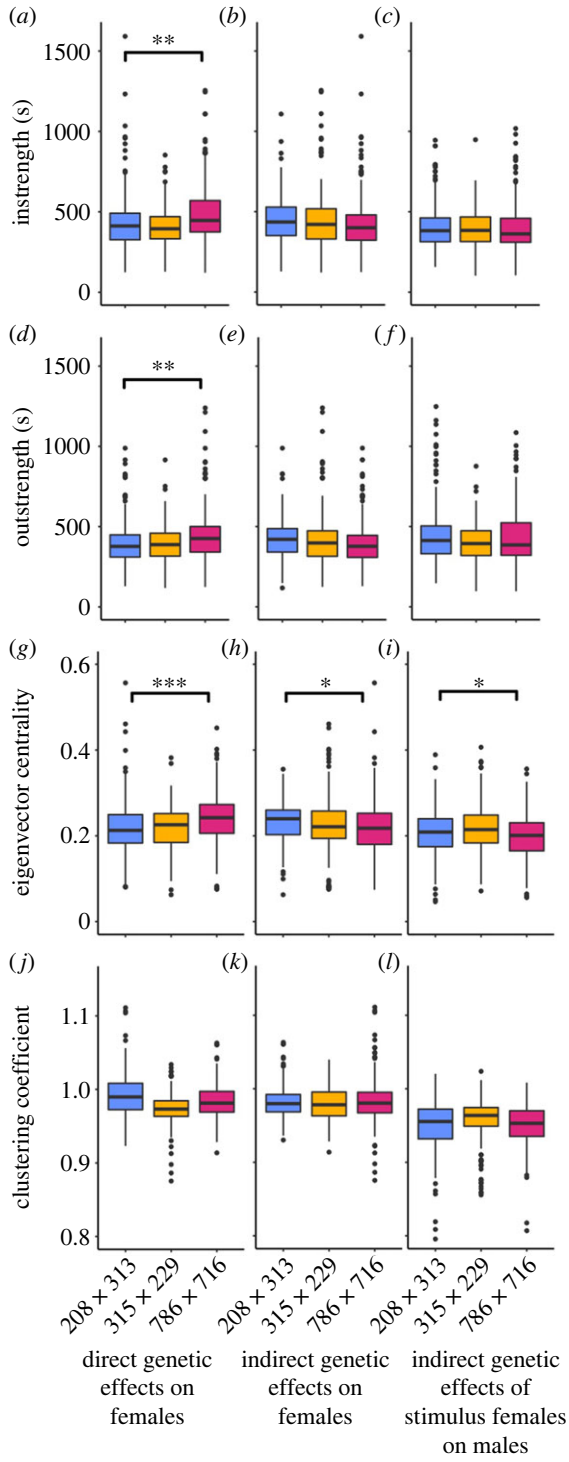

Figure 2. The effects of DGEs (*a*,*d*,*g*,*j*) and IGEs (*b*,*e*,*h*,*k*) on females' network positions, and IGEs of the stimulus genotype males were paired with on males' network positions (*c*,*f*,*i*,*l*). Genotype labels indicate the maternal parent genotype crossed with (x) the paternal genotype to create replicate heterozygous genotypes [52]. Note that genotype numbers are arbitrary and do not describe similarities or differences between genotypes. Boxplots indicate the median, interquartile range (IQR), values within ±1.5× IQR and outliers. Significance of genotypic differences are indicated by asterisks (*< 0.05, **<0.01, ***<0.001). (Online version in colour.)

In contrast to the effects of DGEs and IGEs on females' network positions, we did not find an effect of genotypic *frequency* on females' position in a network (table 1). That is, the network positions of individuals of a particular genotype did not depend on whether there was one individual of that genotype, or nine individuals of that genotype, in the social group. Nor did we find any interaction effects between DGEs, IGEs, and/or a female's genotypic frequency in a social group (table 1).

(b) Indirect genetic effects on male network positions

The genotype of the stimulus females (i.e. the female genotypic majority) in the males' group significantly impacted how central males were to the structure of their social groups (eigenvector centrality, $\chi^2 = 7.827$, $p = 0.020$; figure 2; table 2). While males were generally less central to social group structure compared to females (male eigenvector centrality mean 0.208 ± 0.05 s.d., female eigenvector centrality mean 0.224 ± 0.06 s.d.), the effect of the stimulus female genotype on males' eigenvector centralities also followed our predictions: when males were in a group with a female stimulus genotype with the highest eigenvector centrality values, these males had relatively lower values of eigenvector centrality compared to males who were paired with female stimulus genotypes with lower eigenvector centrality values.

Aside from an IGE of a stimulus female's genotype on males' eigenvector centrality, we found only marginal evidence suggesting IGEs on other aspects of males' network positions. The stimulus female genotype, as well as the specific focal- and stimulus-female genotypic combination, had marginal effects on how cliquish males were (clustering coefficient: stimulus IGE, $\chi^2 = 4.881$, $p = 0.087$; focal IGE-by-stimulus IGE interaction, $\chi^2 = 2.958$, $p = 0.085$; table 2). We found no evidence for IGEs of the stimulus female genotype on how much males engaged in social interactions (instrength and outstrength), IGEs of the focal female genotype on any measure of males' network positions, and no focal IGE-by-stimulus IGE interaction effects on how much males engaged in social interactions or how central males were to the structure of their social groups (instrength, outstrength and eigenvector centrality; table 2).

4. Discussion

To start to understand how IGEs influence social network structure and dynamics, we manipulated the female genotypes present in multiple replicate social groups. Based on prior data, we predicted that a female's own genotype would influence her own eigenvector centrality (i.e. DGEs), and we tested whether those genotypic effects also indirectly influenced the social network positions of other males and females in the network (i.e. IGEs). For females, we found evidence of DGEs for three measures of social network position: instrength, outstrength and eigenvector centrality (table 1, figure 2). We did not see any evidence that the frequency (i.e. one individual or nine individuals) of a particular genotype influenced network position. In both males and females, we found evidence of IGEs for eigenvector centrality, supporting our *a priori* predictions. However, we did not observe IGEs for other aspects of network position (tables 1 and 2). Taken together, our findings show that an individual's position within a social network is dictated not only by its own genotype, but also by the genotypes of other individuals within the network (i.e. DGEs and IGEs). Thus, the genetic basis of network position should extend to include the genotypes of all social group members, at least for some aspects of network position.

Table 1. Model results for direct genetic effects (DGEs), indirect genetic effects (IGEs), genotypic frequency effects and all pairwise combinations of interaction effects on females' social network positions. Italicized values are significant effects, and asterisked values are marginally significant.

social network positions	DGE				IGE				frequency				DGE × IGE				DGE × frequency				IGE × frequency				DGE × IGE × frequency			
	χ^2	d.f.	p	p_R	χ^2	d.f.	p	p_R	χ^2	d.f.	p	p_R	χ^2	d.f.	p	p_R	χ^2	d.f.	p	p_R	χ^2	d.f.	p	p_R	χ^2	d.f.	p	p_R
instrength	5.619	2	0.060	*0.001*	2.310	2	0.315	0.190	285.024	1	<0.001	0.625	1591.231	1	<0.001	0.221	2.667	2	0.264	0.645	1.769	2	0.413	0.859	0.647	1	0.421	0.115
outstrength	3.202	2	0.202	*0.001*	0.524	2	0.770	0.456	365.569	1	<0.001	0.506	1260.671	1	<0.001	0.252	0.563	2	0.755	0.949	1.227	2	0.541	0.824	1.301	1	0.254	0.107
eigenvector centrality	13.095	2	0.001	*<0.001*	3.270	2	0.195	*0.016*	0.315	1	0.575	0.621	0.082	1	0.775	0.753	2.216	2	0.330	0.405	0.689	2	0.709	0.759	2.576	1	0.109	0.108
clustering coefficient	12.149	2	0.002	0.503	11.407	2	0.003	0.121	0.150	1	0.699	0.671	0.285	1	0.593	0.578	4.639	2	0.098	0.052*	1.078	2	0.583	0.611	0.756	1	0.385	0.557

The idea that an individual's position within a social network is dependent not only on its own genotype, but also on the genotypes of other individuals within a network, is perhaps intuitive because an individual's position within a network is dependent on both its own direct social interactions and indirect interactions amongst groupmates [1,2,32,33]. However, studying how DGEs and IGEs affect network structure has remained challenging [4–6,17,40], meaning that we lack empirical support for this hypothesis. Previous studies investigating the heritability of measures of network position have estimated the direct genetic contributions to network position phenotypes, but have often done so using individuals of known genotype or relatedness nested within social networks of individuals of unknown genotypes or relatedness ([37,44,45]; but see [46]). Because of the challenges of controlling for the genetic identities of all individuals within a social group, it has remained unclear to what degree an individual's own genotype and the genotypes of their social groupmates affect each individual's position within a social network.

Our approach partially overcomes this challenge by manipulating and replicating the genotypic composition of groups, and studying the resulting social networks. While *D. melanogaster* flies may not experience groups containing multiple genetically identical individuals in nature, the aim of our study was less about creating social groups that are representative of variation found in the wild, and more about taking an initial step toward integrating IGEs and social network theory. Indeed, many of the individuals in our social groups (all males, and 9/10 females in each group) were genotypically identical, which is not likely to be representative of most wild groups in a species with limited population structure. Exceptions to this include haplodiploid social Hymenoptera (e.g. ants, bees and wasps), which alternatively, are classic examples of how relatedness affects social group dynamics [93]. Additional parallels to this study design are social groups comprised of heterospecific individuals, such as mixed-species groups. Heterospecific social groups comprised of a variable mix of similar and dissimilar individuals can result in groups with variable social and community structures [94–97].

Similarly, our study manipulated only genotypic variation in females, while males were kept genotypically constant. While it is likely that both sexes shape and affect the structure of their social groups in both similar and novel ways, manipulating both sexes could introduce confounding variation about which specific genotypic and sex combinations affect group structure [15,48] and this was not the goal of our current study. Future studies could extend these findings by manipulating males either separately or factorially with females in studies of IGEs and individual effects on social group structure. However, extending our approach to multiple (i.e. more than three) genotypes, and both sexes, in a factorial design with sufficient replication will be difficult, even in organisms amenable to laboratory manipulation such as flies. Creative approaches are needed to move us beyond studying IGEs in dyadic contexts and averaging the effects of social groupmates, into providing a fuller understanding of how social effects cascade through networks of individuals to produce the observed emergent structure of social groups [5].

While we were able to control for prior experience and the genotypic composition of social groups, the specific behaviours that individuals engaged in remain unknown. The genotype-to-network position phenotype relationship is

Table 2. Model results for indirect genetic effects (IGEs) and social group composition effects on males' social network positions. Values in italics indicate significant effects and asterisked values are marginally significant.

	IGE: stimulus female genotype			IGE: focal female genotype			stimulus × focal female genotype		
social network positions	χ^2	d.f.	*p*	χ^2	d.f.	*p*	χ^2	d.f.	*p*
instrength	0.622	2	0.733	0.675	2	0.713	0.077	1	0.781
outstrength	2.153	2	0.341	0.914	2	0.633	0.357	1	0.550
eigenvector centrality	7.827	2	*0.020*	0.366	2	0.833	0.350	1	0.554
clustering coefficient	4.881	2	0.087*	0.484	2	0.785	2.958	1	0.085*

complex, and how the behaviours of individuals and social partners are nested within this relationship can manifest in many ways. For example, in wire-tailed manakins (*Pipra filicauda*), higher territoriality causes males to be more central to network structure (i.e. high eigenvector centrality; [68]). However, in other systems, it seems plausible that highly territorial individuals would be *less* central to group structure (i.e. low-eigenvector centrality) if movement and exploratory behaviour are critical to engaging in social interactions. Our findings of DGEs for measures of network position do translate well to findings of individual differences in other complex social behaviours (i.e. social roles, social responsiveness, social complexity, and social niche specialization; [12,98–100]). Individuals have been shown to consistently vary in network position across both time [63,64,101–103] and contexts [104–108], with individuals' behaviours often being tied to their positions within a social group [19,29,35,36,39,41,43,109]. Correspondingly, individuals have also been shown to plastically alter their network positions in response to prior experience [35,41,43,110], behavioural changes within their social group [108], and perturbations such as demographic changes (reviewed in [111]) and disease prevalence [112].

Such relationships between an individual's genotype, its behaviour, and its network position are complex, and are likely dependent upon multiple behaviours of not just one individual, but the actions and behaviours of conspecifics in a network. For example, even if focal individuals were to behave identically across different social groups, variability in the behaviour and social interactions of their social partners could profoundly influence those focal individuals' network positions. Alternatively, focal individuals may adjust their behaviours to compensate for variation in their networks, thereby maintaining a consistent network position. In other words, apparent DGEs may be driven wholly by IGEs, and *vice versa*. Further, the behavioural mechanisms that give rise to variation in network positions might differ across contexts, study systems or even genotypes. This is perhaps one of many reasons why prior estimates of the genetic contributions to measures of network position have had conflicting results [37,40,44–46]. Future work should continue to link genotypic differences in specific behaviours (or potentially other traits) to DGEs and IGEs for network structure. It will be necessary to address these questions across a range of contexts and study systems in order to come to generalizable conclusions about the quantitative genetic basis of social group structure.

Our lack of finding significant effects of genotypic frequency, DGE-by-genotypic frequency effects, or an IGE-by-genotypic frequency effect (table 1) suggests that the DGEs and IGEs we found do not depend on whether a female is the singular (minority) focal genotype or the stimulus (majority) genotype. These results imply that the presence of a single individual from a particular genotype can impact the social structure of others in their group, and further underscore the potential problems with using the average of all social partners' trait values in IGE analyses. This finding further highlights the need to understand the causes of a group's genotypic composition. For example, genotypes may vary in which type(s) of social environments they choose to experience, generating genotype–social environment correlations [28,49,113–116]. If unaccounted for, such links between genotype and group composition can further obscure how DGEs and IGEs manifest into differences in network position. Combining studies of genetic variation in social group choice with studies of the genetic basis of social network positions will help to reveal the reasons why particular genotypes end up in groups together, and the behavioural and evolutionary consequences of these genetically influenced group formation processes.

Social network analysis has emerged as a well-suited tool for resolving how direct and indirect interactions affect how group structure evolves, as it provides a consistent framework in which social interactions and the genotypes of social group members can be studied simultaneously. However, studies employing both network analysis and IGEs remain scarce, despite their potential to help resolve unanswered questions about how variation in social group structure arises and evolves. The work presented here is a first step toward integrating IGEs and network theory. Future work should continue to leverage this integration, to resolve questions about how variation in DGEs and IGEs shapes the structure and evolution of social groups.

Data accessibility. The data are provided in electronic supplementary material [117].

Authors' contributions. E.W.W.: conceptualization, formal analysis, investigation, methodology, validation, visualization, writing—original draft, writing—review and editing; J.B.S.: conceptualization, funding acquisition, methodology, project administration, supervision, writing—original draft, writing—review and editing.

Both authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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