

REVIEW

The neurogenetics of group behavior in *Drosophila melanogaster*

Pavan Ramdya^{1,*}, Jonathan Schneider^{2,*} and Joel D. Levine^{2,*}

ABSTRACT

Organisms rarely act in isolation. Their decisions and movements are often heavily influenced by direct and indirect interactions with conspecifics. For example, we each represent a single node within a social network of family and friends, and an even larger network of strangers. This group membership can affect our opinions and actions. Similarly, when in a crowd, we often coordinate our movements with others like fish in a school, or birds in a flock. Contributions of the group to individual behaviors are observed across a wide variety of taxa but their biological mechanisms remain largely unknown. With the advent of powerful computational tools as well as the unparalleled genetic accessibility and surprisingly rich social life of *Drosophila melanogaster*, researchers now have a unique opportunity to investigate molecular and neuronal determinants of group behavior. Conserved mechanisms and/or selective pressures in *D. melanogaster* can likely inform a much wider phylogenetic scale. Here, we highlight two examples to illustrate how quantitative and genetic tools can be combined to uncover mechanisms of two group behaviors in *D. melanogaster*: social network formation and collective behavior. Lastly, we discuss future challenges towards a full understanding how coordinated brain activity across many individuals gives rise to the behavioral patterns of animal societies.

KEY WORDS: *Drosophila melanogaster*, Collective behavior, Group behavior, Neurogenetics, Social networks

Introduction

Animals cooperate to feed, mate, outcompete others and thrive in resource-limited environments. When individuals work together, they can accomplish far more than they could in isolation. For example, ants build complex colonies that span many kilometers (Hölldobler and Wilson, 1990) and fish aggregate into large schools that can dissuade (Ioannou et al., 2012) or facilitate escape from predators (Rosenthal et al., 2015). Although we now have a good understanding of behavioral algorithms that give rise to group action (Rogers et al., 2003), very little is known about their underlying neurogenetic mechanisms. This is partly due to the complexity of natural environments: constantly changing visual, auditory, olfactory, gustatory and tactile features make it difficult to determine which cues and biological sensors serve to link individuals with one another. Nevertheless, uncovering these neurogenetic mechanisms remains an important goal as they represent the substrate through which group behaviors likely evolve. There are at least two intertwined facets of group behavior

that are ripe for neurobiological investigation: the formation of social networks and the regulation of collective behavior.

Social networks

In animal groups, individuals can be drawn toward or away from one another for a variety of reasons. For example, long-lasting bonds may exist between family members. On shorter time scales, individuals may be sexually attracted to one another, or to environmental resources such as food patches (Ramos-Fernández et al., 2006). Individuals may also avoid one another because of social or sexual competition, or to maintain a comfortable distance from their neighbors (Simon et al., 2011). The process of satisfying these opposing forces gives rise to a dynamic group-level structure called a social network (Kossinets and Watts, 2006). Social networks have important implications for understanding how information, contagions or behavioral patterns are transmitted among group members (Laland and Plotkin, 1990; Loehle, 1995; Zeltner et al., 2000). Importantly, social networks are descriptive in nature: they do not address the intentionality of inter-individual associations. Even unintentional interactions can facilitate the transmission of disease vectors and important information.


Social networks can be visualized as graphs with nodes (individuals) linked to one another through edges (associations). These links can represent a variety of different kinds of interactions. For example, human social network links can represent how much individuals intend to associate with one another (Apicella et al., 2013), or how much they communicate by email (Kossinets and Watts, 2006). Because non-human animals cannot report their associations, their social networks are typically inferred by measuring whether individuals spend more time with one another than would be expected by chance (Krause et al., 2009; Pinter-Wollman et al., 2013; Williams and Lusseau, 2006). Links within an animal group's social network can be consistent across time because of individually constrained biological roles (e.g. castes in ant colonies) or relatively permanent social roles with impermanent actors (e.g. birds taking turns as alarm-calling sentinels). However, associations can also change over time. Network dynamics can be analyzed using graph-theoretical approaches (Newman, 2010) to understand how group structures change over time (Kossinets and Watts, 2006), how groups constrain individual actions (Centola, 2010) and how manipulations of an individual can influence the group (Williams and Lusseau, 2006).

Collective behavior

A second prominent context for studying the neurogenetics of group behavior is that of collective behavior: coordinated decisions, actions and movements that are not dictated by a central planner but, instead, emerge via distributed interactions among group members (Couzin, 2009; Sumpter, 2006). This distributed mechanism has been effective at explaining collective behaviors across a wide range of taxa and models including human crowds (Silverberg et al., 2013; Turner and Killian, 1957), fish schools (Berdahl et al., 2013; Rosenthal et al., 2015; Ward et al., 2011), locust swarms (Bazazi

¹Department of Biology and Bioengineering, California Institute of Technology, Pasadena, CA 91106, USA. ²Department of Biology, University of Toronto at Mississauga, Mississauga, Ontario, Canada L5L1C6.

*Authors for correspondence (pavan.ramdya@epfl.ch; j.schneider@utoronto.ca; joel.levine@utoronto.ca)

 P.R., 0000-0001-5425-4610; J.S., 0000-0003-1114-618X; J.D.L., 0000-0002-6254-6274

et al., 2008; Buhl et al., 2006), ant colonies (Gelblum et al., 2015; Khuong et al., 2016), bird flocks (Cavagna et al., 2010; Nagy et al., 2010), midge swarms (Attanasi et al., 2014), worms (Yuan et al., 2014), amoebae (Gregor et al., 2010), cells (Vedel et al., 2013), robots (Giomi et al., 2012; Rubenstein et al., 2014) and colloids (Bricard et al., 2014). In animals, group-level behaviors likely evolved under very specific ecological and social conditions (Gordon, 2014; Hein et al., 2015) to facilitate foraging (Berdahl et al., 2013; Handegard et al., 2012) and predator avoidance (Ioannou et al., 2012).

A framework for investigating group behaviors

Social networks and collective behavior share important features. As mentioned earlier, in both cases, group dynamics result from distributed interactions among individuals rather than coordination by a central planner. Consequently, mechanistic investigations of these phenomena have focused on inter-individual transmission of social signals. For example, studies of eusocial insects such as ants have identified tactile and indirect chemical signals that are used to coordinate brood raising, colony defense, food foraging and nest building (Hölldobler and Wilson, 1990; Khuong et al., 2016). Similarly, the collective, ordered movements of *Notemigonus crysoleucas* fish schools have been shown to emerge from the propagation of visual social cues across individuals in a population (Berdahl et al., 2013).

At first glance, this paradigm, in which group dynamics emerge from a set of inter-individual interaction rules, lends itself to well to reductionist studies. Typically, one first records and quantifies a group behavior. To accomplish this, one must first be able to accurately track the movements of individuals. Accurate identification of each individual is vital for the study of social networks but may be less crucial for understanding collective behavior. Then, to infer interaction rules giving rise to the group-level phenomenon, individuals, pairs and even triads (Katz et al., 2011) of animals are closely studied in highly controlled conditions. It should be noted that this reductionist approach, while highly informative, has limitations. A focus on dyadic/triadic interactions cannot directly interrogate emergent group-level properties, making it difficult to predict how group dynamics would be modified by the altering pair-wise interactions (Schneider et al., 2012a, 2016). For example, the structure of a *Lasius niger* ant colony nest depends on the intensity and decay rate of pheromone deposits (Khuong et al., 2016). This relationship is a complex function that cannot simply be intuited. Therefore, computational models have become indispensable to test the sufficiency of interaction rules intended to explain group dynamics. Specifically, the average individual response to social or environmental cues is measured and then used to control the motion of simulated agents. These simulations can help to test the sufficiency of parsimonious behavioral mechanisms underlying group dynamics. Although the algorithms used in simulations do not necessarily reflect exact biological solutions, they help to constrain the number of possible models and generate predictions to be tested in future experiments performed with real animals.

Determining inter-individual interaction rules can also provide a natural entry point for identifying the neurogenetic underpinnings of group behavior (e.g. neurons and genes used to send and receive social signals). To do this, one must be able to manipulate and measure the activity of neurons and genes in behaving groups of animals to measure the effect of experimental perturbations on the topology of social networks or the dynamics of collective behavior. Although advances in gene-editing technology (Wright et al., 2016)

now make it possible to study how genes and neurons govern group behavior in classic models such as ant colonies (Libbrecht et al., 2013), locust swarms and bird flocks, these approaches are still under development. However, many exciting questions can already be addressed using genetic model organisms such as fish (e.g. *Danio rerio*; Miller and Gerlai, 2012; Wyatt et al., 2015) and flies (e.g. *Drosophila melanogaster*; Clark et al., 2013; Lihoreau et al., 2016). In this Review, we focus on studies of group behavior in *Drosophila melanogaster*.

Group behavior in *Drosophila melanogaster*

Drosophila melanogaster has long been an experimental workhorse, enabling important advances in multiple areas of biology (Laland and Plotkin, 1990; Lemaitre et al., 1996; Loehle, 1995; Morgan, 1910; Muller, 1927; Nüsslein-Volhard and Wieschaus, 1980). Although much of what we know about *D. melanogaster* is inferred from laboratory studies, diverse *Drosophila* species are spread throughout the world and are known to have a rich natural ecology (Carson and Kaneshiro, 1976; Markow, 2015). *Drosophila melanogaster* is rarely considered a model for group sociality, but this commonly held view is slightly misleading. Flies form groups on food and oviposition sites (Shorrocks, 1972). This aggregation can be modulated by the distribution of environmental resources, genetic determinants that determine the degree of resource exploitation versus exploration/foraging (Sokolowski, 2010), and social mechanisms. For example, adult males lay pheromones that attract females to food sources (Lin et al., 2015), and larvae deposit aggregation pheromones (Mast et al., 2014) that may be used to facilitate food digestion (Durisko et al., 2014) and create opportunities for cannibalistic interactions (Vijendravarma et al., 2013).

A question that naturally follows the observation that *D. melanogaster* aggregate is whether fly groups benefit individual animals. Evidence suggests that group life may facilitate social learning (Battesti et al., 2012; Mery et al., 2009), influence foraging decisions (Lihoreau et al., 2016) and generate a heightened awareness of environmental stressors (Suh et al., 2004). Therefore, although *D. melanogaster* groups cannot rival the complexity of eusocial insect colonies, they may serve as a useful model for identifying conserved mechanisms for simple emergent group-level behaviors. Here, we highlight two studies using *D. melanogaster* that aimed to uncover sensory genes and neural pathways required for two group phenomena – social networks (Schneider et al., 2012b) and collective behavior (Ramdya et al., 2015).

Drosophila social networks

Schneider et al. (2012b) combined graph theoretical analyses (Boccaletti et al., 2006; Pinter-Wollman et al., 2013) and semi-automated behavioral tracking (Branson et al., 2009) to quantify *D. melanogaster* social networks and to identify sensory modalities and genes that influence network structure. Briefly, they tracked the behaviors of groups of flies within a shallow circular arena (Fig. 1A) (Simon and Dickinson, 2010). They then quantified *D. melanogaster* social interaction networks (SINs) by building a behavioral classifier that identified when pairs of flies were within two body lengths of one another and oriented such that one fly could function as an interactor and the other as an interactee (Fig. 1B,C). Although these criteria were determined empirically, new approaches can perform this task in an automated manner (Schneider and Levine, 2014). Schneider et al. (2012b) then recorded these interactions over 30 min. A sliding window approach was used for each experiment to gain a dynamic view of social networks; once a network accumulated a critical density, the initial

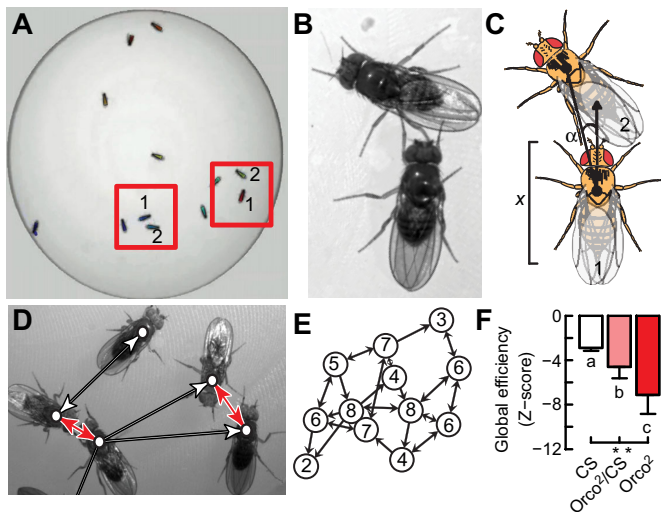


Fig. 1. *Drosophila melanogaster* social interaction networks. (A) Still frame from a video used to empirically determine a social interaction. Twelve flies are video-recorded and tracked in a circular arena. Examples of ongoing interactions are highlighted (red boxes) in which interactor flies are labeled 1 and recipients of interactions (interactees) are labeled 2. (B) High-resolution image of flies interacting in an arena. (C) Criteria used to define an interaction. The interactor (1) must be within two body lengths (x) of the interactee (2). Additionally, the angle between the long axis of the interactor and the line linking the two flies (α) must be less than 90 deg. (D) Interactions between flies (red arrows) are recorded and added to a history of prior interactions (white arrows) to build a social interaction network (SIN). Directionality of the arrows indicates the identities of the interactor (origin) and interactee (arrowhead). (E) Example of a single network iteration for a SIN. Each circle is a node representing an individual fly. Arrows indicate the directionality of observed interactions between each fly. Each fly's degree (number of interactions) is superimposed upon each node. (F) SIN measures are influenced by chemosensory cues. Olfactory-defective homozygous mutants *Orco*² displayed lowered global efficiency than control flies ($P < 0.01$). Groups are color-coded: Canton-S ($n=43$, white), *Orco*²/Canton-S ($n=24$, pink), *Orco*²/*Orco*² ($n=14$, red).

interaction was ignored and a new network was created from the second interaction onward in an iterative manner – this collection of networks captures how a SIN changes through time (Fig. 1D,E). The authors confirmed that SINs are not simply artifacts resulting from the geometry of the experimental arena and/or a consequence of flies randomly encountering one another. They then quantified SINs using the common graph theory metrics of assortativity, clustering, betweenness centrality and efficiency, and found that network properties were stable (i.e. observed reproducibly) throughout each experiment. Clear differences in network properties could be found among genetically distinct wild-type *D. melanogaster* strains. Intriguingly, social network properties have also been found to be heritable in humans (Fowler et al., 2009).

Finally, Schneider et al. (2012b) asked which sensory modalities shape inter-fly interactions and SINs. Impairments of vision and hearing did not significantly affect SIN organization. Flies are coated with cuticular hydrocarbon pheromones – a chemical signature of their sex, species and mating state (Billeter et al., 2009). Therefore, hydrocarbon chemosensing was a likely candidate for coordinating inter-fly interactions. To test the role of taste-dependent hydrocarbon sensing, the authors studied flies with mutations in the *poxn* gene. This mutation causes sensilla that are normally destined to convey taste to transform into those that transmit mechanosensory signals (Awasaki and Kimura, 1997). They observed that *poxn* mutants have SINs that are statistically

indistinguishable from their spatiotemporally shuffled control networks. The authors interpreted this as demonstrating that gustation is required for the non-random structure of *D. melanogaster* social networks. Finally, to test the influence of long-distance odor cues in social network structure, the authors impaired olfaction by mutating *Orco*, a gene that encodes an important olfactory co-receptor in *Drosophila* (Larsson et al., 2004). They observed a strong effect on social networks, supporting a role for olfactory chemosensing in the organization of SINs (Fig. 1F).

By combining quantitative network analysis with genetic tools, the study of Schneider et al. (2012b) provides a first look at sensory genes that contribute to the organization of social networks in an animal group. Still, many challenges lie ahead in interpreting *D. melanogaster* social networks. First, the relative influences of individual behavioral tendencies, life histories and initial experimental conditions on the topology of social networks remains unclear. Second, graph theoretical metrics have their roots in the study of information flow (Noble et al., 2004; Wu et al., 2004). Connected individuals in a social network have more frequent contact with one another, a prerequisite for communicating some kinds of information. Generally speaking, information communication among animals has been difficult to quantify. Therefore, to what extent and how information flows in a meaningful way through *D. melanogaster* social networks remains to be determined (however, see Battesti et al., 2012 for sequential transfer of information about oviposition site preference). Finally, unlike in laboratory experiments, the natural world is full of constantly changing group membership and sensory cues. Therefore, whether SINs can establish themselves in nature is unknown. If they do, how SINs remain resilient in the face of stimuli that drive goal-oriented behaviors such as feeding, mating and predator escape has yet to be determined.

Drosophila collective behavior

The collective escape of fish schools and stampeding of human crowds are vivid examples of how strong sensory cues can modify and disrupt group structures. The time course of these escape behaviors has been well studied, revealing that social interactions rather than environmental cues largely determine how collective actions unfold (Rosenthal et al., 2015). Still, neurons and genes that regulate these interactions remain largely unknown. To address this gap, Ramdya et al. (2015) investigated the neurogenetic mechanisms underlying collective behavior in *D. melanogaster*.

As in the study of *D. melanogaster* social networks (Schneider et al., 2012b) and collective behavior studies in other species (Attanasi et al., 2014; Berdahl et al., 2013), the authors of Ramdya et al. (2015) used semi-automated tracking methods (Branson et al., 2009; Ramdya et al., 2012) to quantify animal behavior in a simple behavioral arena. They asked to what extent group density influences the odor avoidance decisions of individual flies. Specifically, an aversive odorant, 5% CO₂, was passed through one half of a rectangular arena while air was passed through the other half (Fig. 2A). Flies in the odor zone were tracked and the time they spent in the air zone was used to quantify olfactory decision-making. The authors were surprised to find that individual odor avoidance increased when animals were placed in groups of densities similar to those found on a food source (Ramdya et al., 2015) – a hallmark of collective behavior.

Previous studies of ordered group behavior support the notion that collective behaviors arise from interactions among group members (Couzin, 2009; Sumpter, 2006). Consistent with this, Ramdya et al. (2015) found that odor-responsive flies seemed to

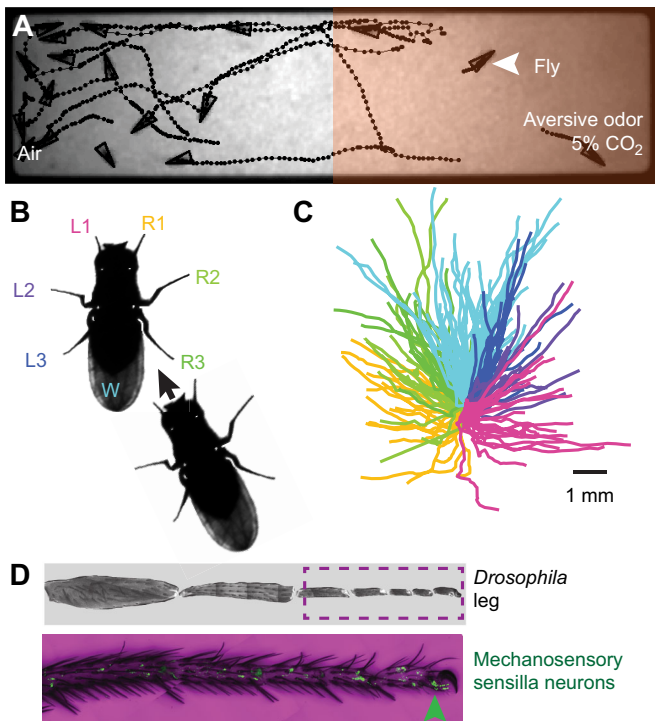


Fig. 2. *Drosophila melanogaster* collective behavior. (A) Collective odor avoidance. An aversive odorant, 5% CO₂, enters the arena on the right side (orange), resulting in odor avoidance of the group to the air zone on the left side. The location and trajectory of each fly is indicated (black triangles and dashed lines). (B) Collective avoidance arises from inter-fly touch-evoked walking reactions or encounter responses in which a moving animal (bottom) touches a stationary animal (top). (C) Touch-evoked walking trajectories are color-coded by the leg (L1–3, R1–3) or wing (W) touched (>100 trajectories are overlaid). (D) Touch-evoked walking reactions require mechanosensory sensilla neurons located on the distal tarsal segments of the legs (bottom; green cell bodies).

elicit walking reactions from otherwise non-responsive flies by touching their legs or wings (Fig. 2B). In groups of high enough density, these newly activated animals would then interact with other, sedentary animals. This led to a positive feedback cascade and an en masse escape from the odor zone – an effect remarkably similar to collective escape in fish schools (Rosenthal et al., 2015) and human crowds (Helbing et al., 2000). Using an agent-based computer simulation they found that suppressing inter-fly touch responses – termed ‘encounter responses’ – could abolish *in silico* collective behavior.

To test this *in silico* prediction in real animals, they next identified genetic and neural pathways that convey inter-fly touch-mediated encounter responses. First, they discovered that artificial mechanical stimulation of flies with a metallic disk is sufficient to drive encounter-response-like walking. Touch-evoked walking kinematics depend on the appendage that was touched (Fig. 2B,C) in a manner strikingly similar to the kinematics of inter-fly encounter responses and reminiscent of escape behaviors in cockroaches (Schaefer et al., 1994). This suggested that mechanosensing might be responsible for encounter responses. Therefore, they performed a screen of candidate mechanosensory genes to identify those responsible for relaying tactile information to the nervous system. The authors discovered that the gentle touch sensor, NOMPC (Yan et al., 2012), is required for inter-fly encounter responses. Next, to identify mechanosensory neural pathways that convey touch information from the sensory periphery to the central nervous system, they performed a neural inactivation screen. Mechanosensory sensilla neurons that decorate

the tarsus, a distal region of the fly’s legs (Fig. 2D), were crucial for encounter responses. Optogenetic activation of these mechanosensory neurons with light caused flies to produce encounter-response-like walking.

Having identified an important role for the *nompC* gene and mechanosensory sensilla neurons in eliciting encounter responses, the authors next tested the prediction that encounter responses would be crucial for collective odor avoidance. Indeed, they observed that individuals in groups do not avoid the odor better than isolated individuals for *nompC* mutants, or groups of flies with silenced mechanosensory sensilla neurons. These results link specific mechanosensory neurons and a mechanosensory gene to *D. melanogaster* collective behavior.

Finally, to firmly establish a causal relationship between touch-evoked walking and collective odor avoidance, Ramdya et al. (2015) asked whether flies that were unable to sense the noxious odorant could be herded out of the odor zone by interactions with wild-type flies. To do this, they measured odor avoidance in transgenic flies that were unable to smell the noxious odorant. Although these flies exhibited almost no odor avoidance when alone, they showed high odor avoidance when placed in groups with normal, wild-type flies. This suggested that inter-fly touch is sufficient to convey behaviorally relevant information from one animal to another in a way resembling the physical interactions that drive stampedes in human crowds (Helbing et al., 2000; Moussaid et al., 2011).

Shared features of *Drosophila* group behaviors

These two studies (Ramdya et al., 2015; Schneider et al., 2012b) illustrate how quantitative behavioral tracking and genetic tools can be combined to identify neurogenetic pathways that sculpt the dynamics of animal groups. Social networks and collective motion likely represent intertwined facets of group behavior: networks characterize relatively stable relationships between group members while collective motion describes how these groups react to powerful sensory cues or the spontaneous actions of individual group members. Mechanistically, tactile and close-range gustatory cues are both important for structuring social networks (Schneider et al., 2012b), while tactile interactions alone appear to coordinate collective actions (Ramdya et al., 2015). These studies highlight the importance of touch in the social life of the fly, but they have only begun to scratch the surface of how group-level behaviors are orchestrated by the nervous system.

Individuality and the group

One of the most fascinating mysteries arising from these studies concerns the origin of behavioral heterogeneity among group members. In social networks, individuals can be more or less connected within a group. The origins of these differences may be intrinsic (e.g. due to innate tendencies) and/or extrinsic (e.g. due to current location). Similarly, although the analysis of collective behavior often assumes that each group member is interchangeable, differences between individuals likely determine who will initiate a cascade of group motion (Rosenthal et al., 2015). While it is difficult to determine the extrinsic contributions to these differences, measuring the contribution of innate tendencies can give us an indirect estimate of social influences. This can be accomplished by repeatedly testing specific individuals in different group experiments. In principle, the requisite technologies exist: one can repeatedly identify freely behaving animals based on morphological features (Pérez-Escudero et al., 2014) and automatically pick and place them using robotic technologies that

are currently under development (Savall et al., 2015). If individuals exhibit reproducible roles in groups across multiple experiments, bioinformatics approaches might then be used to identify the genetic determinants of high network connectivity, or leadership in collective motion.

Accident or intent

A thornier question relates to the ethological interpretation of *D. melanogaster* group behaviors. It is tempting to treat flies like simple automata that respond deterministically to short-time-scale environmental and social cues. We know that the behaviors of even simple robots can appear to be complex, motivated and intentional (Braitenberg, 1986; Walter, 1950). This raises the question of to what extent we can ascribe intentionality to interactions between *D. melanogaster* group members as they build social networks and participate in collective motion. These behaviors may simply be epiphenomena that arise from random inter-fly interactions. We would argue that, regardless of whether social networks and collective behavior are intentional or accidental, if they play significant, well-defined roles in the individual's life, their investigation offers useful insight into the social workings of the fly. Although it is very difficult to directly interrogate the intentionality of fly behavior, a suitable proxy might be found in testing whether walking trajectories are enriched in social encounters when compared with random null models or shuffled trajectories (Schneider et al., 2012b). Additionally, one can use robots or artificial flies (Agrawal et al., 2014; Zabala et al., 2012) to further identify and manipulate key sensory features – hydrocarbon profiles, shapes, colors and odors – that draw flies to one another, making group behaviors possible (Bartelt et al., 1985; Lin et al., 2015).

As simple as possible, but not simpler

In the laboratory, there is an unavoidable trade-off between achieving experimental control and preserving ecologically relevant features. Both of the highlighted studies (Ramdya et al., 2015; Schneider et al., 2012b) were performed in simple arenas, raising the question of how group behaviors would differ in the fly's complex, natural environment. For example, in both studies, groups consisted only of members of one sex. In the presence of both males and females, courtship and mating behaviors would likely influence SIN topologies. During courtship, male flies emit songs via wing vibrations, tap and lick (Yamamoto and Koganezawa, 2013). The presence of females may also incite aggression among males, causing them to posture, lunge and box (Zwarts et al., 2012). Therefore, it is likely that courting or fighting males would transiently become tightly linked with courted females or sparring partners in mixed-sex SINs. Courtship behaviors might also affect and perhaps even diminish the initiation of collective motion by habituating courted females and fighting males to tactile stimulation. To understand the influences of other social interactions on group behaviors, one could track both individual movements and ongoing social behaviors using machine-vision tools such as JAABA (Kabra et al., 2012).

These two studies also made use of a relatively simple stimulus space, although we know that *Drosophila* naturally aggregate on food and oviposition sites (Shorrock, 1972) rich in tactile, visual, olfactory and gustatory cues. The effects of these other sensory features on group behavior are difficult to anticipate; even in simple laboratory environments, the structure of social networks and the unfolding of collective motion are largely unpredictable (Ramdya et al., 2015; Schneider et al., 2012b). For example, on the one hand, touch-evoked encounter responses might be suppressed if a hungry

animal is grazing on a food patch. On the other hand, there might be an increase in the likelihood of inter-fly encounters as animals are mutually attracted to light (phototaxis), food odors (chemotaxis) and edges (thigmotaxis). Similarly, steady-state sensory cues would almost certainly sculpt social network topology by shifting the distribution of animals in space. To address these influences of these environmental cues on group social behaviors, animals would have to be studied in more ethologically relevant arenas. Visual occlusions could be overcome by tracking behavior using multiple cameras (Straw et al., 2011). The importance of different environmental cues could be determined using remote thermogenetic silencing approaches to shut down sensory neurons in freely behaving flies (Bath et al., 2014).

Conserved mechanisms and common approaches

As we embark on more and more complex studies of *D. melanogaster* group behavior, one concern is to what extent discoveries made in the fly can inform our understanding of other classically social taxa such as ants, fish, birds and humans. There are a number of reasons to be optimistic. First, invertebrates share many homologous genes and neural subtypes, making it likely that mechanisms for *D. melanogaster* may have direct corollaries in other invertebrates. For example, ants use antennation to communicate with one another, a process that depends on both tactile and chemosensory signaling (Hölldobler, 1999). As mentioned earlier, these two modalities are crucial for *D. melanogaster* social network formation and collective behavior (Ramdya et al., 2015; Schneider et al., 2012b). Similarly, in locusts, tactile stimulation of the legs is responsible for the transition from solitary to gregarious states, resulting in the formation of massive collectively marching bands (Rogers et al., 2003; Simpson et al., 2001). Touch interactions also direct the dynamics of dense human crowds (Moussaïd et al., 2011). It should be noted that even in cases where the biological mechanisms for *D. melanogaster* do not directly inform classic models of group behavior, the scientific framework for cracking these problems is likely very similar. Therefore, studies using flies can demonstrate how to deeply investigate the neurogenetic mechanisms of group behavior in other species when the requisite tools become available.

A measure of whether we have a clear picture of what drives group dynamics will be whether we can predict how group behaviors unfold under arbitrary biological and environmental conditions. Because the underlying mechanisms range across multiple scales – molecular to circuit-level to behavioral – making such a prediction will require knowing the initial positions of each individual, their long- and short-term behavioral tendencies, gene expression levels, neural states, the make-up of the sensory environment and inter-individual behavioral interaction rules. However daunting the prospect of accumulating and making sense of all of this data, the fruits of this labor promise to be sweet: a deep understanding of group behavior in *D. melanogaster* may provide a general framework for how coordinated brain activity across many individuals gives rise to the fascinating behavioral patterns of animal societies.

Competing interests

The authors declare no competing or financial interests.

Funding

P.R. was supported by a Swiss National Science Foundation (Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung) Fellowship (P300P3_158511); J.S. and J.D.L. acknowledge support from the Canadian Institutes of Health Research, the Natural Sciences and Engineering Research Council of Canada and the Canadian Institute for Advanced Research.

References

- Agrawal, S., Safarik, S. and Dickinson, M.** (2014). The relative roles of vision and chemosensation in mate recognition of *Drosophila melanogaster*. *J. Exp. Biol.* **217**, 2796–2805.
- Apicella, C. L., Marlowe, F. W., Fowler, J. H. and Christakis, N. A.** (2013). Social networks and cooperation in hunter-gatherers. *Nature* **481**, 497–501.
- Attanasi, A., Cavagna, A., Del Castello, L., Giardina, I., Melillo, S., Parisi, L., Pohl, O., Rossaro, B., Shen, E., Silvestri, E. et al.** (2014). Collective behaviour without collective order in wild swarms of midges. *PLoS Comput. Biol.* **10**, e1003697.
- Awasaki, T. and Kimura, K.** (1997). *pox-neuro* is required for development of chemosensory bristles in *Drosophila*. *J. Neurobiol.* **32**, 707–721.
- Bartelt, R. J., Schaner, A. M. and Jackson, L. L.** (1985). *cis*-Vaccenyl acetate as an aggregation pheromone in *Drosophila melanogaster*. *J. Chem. Ecol.* **11**, 1747–1756.
- Bath, D. E., Stowers, J. R., Hörmann, D., Poehlmann, A., Dickson, B. J. and Straw, A. D.** (2014). FlyMAD: rapid thermogenetic control of neuronal activity in freely walking *Drosophila*. *Nat. Methods* **11**, 756–762.
- Battesti, M., Moreno, C., Joly, D. and Mery, F.** (2012). Spread of social information and dynamics of social transmission within *Drosophila* groups. *Curr. Biol.* **22**, 309–313.
- Bazazi, S., Buhl, J., Hale, J. J., Anstey, M. L., Sword, G. A., Simpson, S. J. and Couzin, I. D.** (2008). Collective motion and cannibalism in locust migratory bands. *Curr. Biol.* **18**, 735–739.
- Berdahl, A., Torney, C. J., Ioannou, C. C., Faria, J. J. and Couzin, I. D.** (2013). Emergent sensing of complex environments by mobile animal groups. *Science* **339**, 574–576.
- Billeter, J.-C. C., Atallah, J., Krupp, J. J., Millar, J. G. and Levine, J. D.** (2009). Specialized cells tag sexual and species identity in *Drosophila melanogaster*. *Nature* **461**, 987–991.
- Boccaletti, S., Latora, V., Moreno, Y., Chavez, M. and Hwang, D.** (2006). Complex networks: structure and dynamics. *Phys. Rep.* **424**, 175–308.
- Braitenberg, V.** (1986). *Vehicles: Experiments in Synthetic Psychology*. Cambridge, MA: The MIT Press.
- Branson, K. M., Robie, A. A., Bender, J. A., Perona, P. and Dickinson, M. H.** (2009). High-throughput ethomics in large groups of *Drosophila*. *Nat. Methods* **6**, 451–457.
- Bricard, A., Caussin, J.-B., Desreumaux, N., Dauchot, O. and Bartolo, D.** (2014). Emergence of macroscopic directed motion in populations of motile colloids. *Nature* **503**, 95–98.
- Buhl, J., Sumpter, D. J. T., Couzin, I. D., Hale, J. J., Despland, E., Miller, E. R. and Simpson, S. J.** (2006). From disorder to order in marching locusts. *Science* **312**, 1402.
- Carson, H. L. and Kaneshiro, K. Y.** (1976). *Drosophila* of Hawaii: systematics and ecological genetics. *Annu. Rev. Ecol. Syst.* **7**, 311–345.
- Cavagna, A., Cimarelli, A., Giardina, I., Parisi, G., Santagati, R., Stefanini, F. and Viale, M.** (2010). Scale-free correlations in starling flocks. *Proc. Natl. Acad. Sci.* **107**, 11865–11870.
- Centola, D.** (2010). The spread of behavior in an online social network experiment. *Science* **329**, 1194–1197.
- Clark, D. A., Freifeld, L. and Clandinin, T. R.** (2013). Mapping and cracking sensorimotor circuits in genetic model organisms. *Neuron* **78**, 583–595.
- Couzin, I. D.** (2009). Collective cognition in animal groups. *Trends Cogn. Sci.* **13**, 36–43.
- Durisko, Z., Kemp, R., Mubasher, R. and Dukas, R.** (2014). Dynamics of social behavior in fruit fly larvae. *PLoS ONE* **9**, e95495.
- Fowler, J. H., Dawes, C. T. and Christakis, N. A.** (2009). Model of genetic variation in human social networks. *Proc. Natl. Acad. Sci. USA* **106**, 1720–1724.
- Gelblum, A., Pinkoviezky, I., Fonio, E., Ghosh, A., Gov, N. and Feinerman, O.** (2015). Ant groups optimally amplify the effect of transiently informed individuals. *Nat. Commun.* **6**, 7729.
- Giomi, L., Hawley-Weld, N. and Mahadevan, L.** (2012). Swarming, swirling and stasis in sequestered bristle-bots. *Proc. R. Soc. A Math. Phys. Eng. Sci.* **469**, 1–18.
- Gordon, D. M.** (2014). The ecology of collective behavior. *PLoS Biol.* **12**, e1001805.
- Gregor, T., Fujimoto, K., Masaki, N. and Sawai, S.** (2010). The onset of collective behavior in social amoebae. *Science* **328**, 1021–1025.
- Handegard, N. O., Boswell, K. M., Ioannou, C. C., Leblanc, S. P., Tjøstheim, D. B. and Couzin, I. D.** (2012). The dynamics of coordinated group hunting and collective information transfer among schooling prey. *Curr. Biol.* **22**, 1213–1217.
- Hein, A. M., Rosenthal, S. B., Hagstrom, G. I., Berdahl, A., Torney, C. J. and Couzin, I. D.** (2015). The evolution of distributed sensing and collective computation in animal populations. *Elife* **4**, e10955.
- Helbing, D., Farkas, I. and Vicsek, T.** (2000). Simulating dynamical features of escape panic. *Nature* **407**, 487–490.
- Hölldobler, B.** (1999). Multimodal signals in ant communication. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **184**, 129–141.
- Hölldobler, B. and Wilson, E. O.** (1990). *The Ants*. Cambridge, MA: Harvard University Press.
- Ioannou, C. C., Guttal, V. and Couzin, I. D.** (2012). Predatory fish select for coordinated collective motion in virtual prey. *Science* **337**, 1212–1215.
- Kabra, M., Robie, A. A., Rivera-Alba, M., Branson, S. and Branson, K. M.** (2012). JAABA: interactive machine learning for automatic annotation of animal behavior. *Nat. Methods* **10**, 64–67.
- Katz, Y., Tunstrom, K., Ioannou, C. C., Huepe, C. and Couzin, I. D.** (2011). Inferring the structure and dynamics of interactions in schooling fish. *Proc. Natl. Acad. Sci. USA* **108**, 18720–18725.
- Khuong, A., Gautrais, J., Perna, A., Sbaï, C., Combe, M., Kuntz, P., Jost, C. and Theraulaz, G.** (2016). Stigmergic construction and topochemical information shape ant nest architecture. *Proc. Natl. Acad. Sci. USA* **113**, 1303–1308.
- Kossinets, G. and Watts, D. J.** (2006). Empirical analysis of an evolving social network. *Science* **311**, 88–90.
- Krause, J., Lusseau, D. and James, R.** (2009). Animal social networks: an introduction. *Behav. Ecol. Sociobiol.* **63**, 967–973.
- Laland, K. N. and Plotkin, H. C.** (1990). Social learning and social transmission of foraging information in Norway rats (*Rattus norvegicus*). *Anim. Learn. Behav.* **18**, 246–251.
- Larsson, M. C., Domingos, A. I., Jones, W. D., Chiappe, M. E., Amrein, H. and Vosshall, L. B.** (2004). *Or83b* encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. *Neuron* **43**, 703–714.
- Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J.-M. and Hoffmann, J. A.** (1996). The dorsoventral regulatory gene cassette *spätzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults. *Cell* **86**, 973–983.
- Libbrecht, R., Oxley, P. R., Kronauer, D. J. C. and Keller, L.** (2013). Ant genomics sheds light on the molecular regulation of social organization. *Genome Biol.* **14**, 212.
- Lihoreau, M., Clarke, I. M., Buhl, J., Sumpter, D. J. T. and Simpson, S. J.** (2016). Collective selection of food patches in *Drosophila*. *J. Exp. Biol.* **219**, 668–675.
- Lin, C.-C., Prokop-Prigge, K. A., Preti, G. and Potter, C. J.** (2015). Food odors trigger *Drosophila* males to deposit a pheromone that guides aggregation and female oviposition decisions. *Elife* **4**, e08688.
- Loehle, C.** (1995). Social barriers to pathogen transmission in wild animal populations. *Ecology* **76**, 326–335.
- Markow, T. A.** (2015). The secret lives of *Drosophila* flies. *Elife* **4**, e06793.
- Mast, J. D., De Moraes, C. M., Alborn, H. T., Lavis, L. D. and Stern, D. L.** (2014). Evolved differences in larval social behavior mediated by novel pheromones. *Elife* **3**, e04205.
- Mery, F., Varela, S. A. M., Danchin, E., Blanchet, S., Parejo, D., Coolen, I. and Wagner, R. H.** (2009). Public versus personal information for mate copying in an invertebrate. *Curr. Biol.* **19**, 730–734.
- Miller, N. and Gerlai, R.** (2012). From schooling to shoaling: patterns of collective motion in zebrafish (*Danio rerio*). *PLoS ONE* **7**, e48865.
- Morgan, T. H.** (1910). Sex limited inheritance in *Drosophila*. *Science* **32**, 120–122.
- Moussaïd, M., Helbing, D. and Theraulaz, G.** (2011). How simple rules determine pedestrian behavior and crowd disasters. *Proc. Natl. Acad. Sci. USA* **108**, 6884–6888.
- Muller, H. J.** (1927). Artificial transmutation of the gene. *Science* **66**, 84–87.
- Nagy, M., Ákos, Z., Biro, D. and Vicsek, T.** (2010). Hierarchical group dynamics in pigeon flocks. *Nature* **464**, 890–893.
- Newman, M.** (2010). *Networks*. Oxford: Oxford University Press.
- Noble, J., Davy, S. and Franks, D. W.** (2004). Effects of the topology of social networks on information transmission. In *From Animals to Animats 8: Proceedings of the Eighth International Conference on Simulation of Adaptive Behavior* (ed. S. Schaal, A. J. Ijspeert, A. Billard and S. Vijayakumar), pp. 395–404. Cambridge, MA: MIT Press.
- Nüsslein-Volhard, C. and Wieschaus, E.** (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**, 795–801.
- Pérez-Escudero, A., Vicente-Page, J., Hinz, R. C., Arganda, S. and de Polavieja, G. G.** (2014). idTracker: tracking individuals in a group by automatic identification of unmarked animals. *Nat. Methods* **11**, 743–748.
- Pinter-Wollman, N., Hobson, E. A., Smith, J. E., Edelman, A. J., Shizuka, D., de Silva, S., Waters, J. S., Prager, S. D., Sasaki, T., Wittemyer, G. et al.** (2013). The dynamics of animal social networks: analytical, conceptual, and theoretical advances. *Behav. Ecol.* **1**–14.
- Ramdya, P., Schaffter, T., Floreano, D. and Benton, R.** (2012). Fluorescence Behavioral Imaging (FBI) tracks identity in heterogeneous groups of *Drosophila*. *PLoS ONE* **7**, e48381.
- Ramdya, P., Lichocki, P., Cruchet, S., Frisch, L., Tse, W., Floreano, D. and Benton, R.** (2015). Mechanosensory interactions drive collective behaviour in *Drosophila*. *Nature* **519**, 233–236.
- Ramos-Fernández, G., Boyer, D. and Gómez, V. P.** (2006). A complex social structure with fission–fusion properties can emerge from a simple foraging model. *Behav. Ecol. Sociobiol.* **60**, 536–549.
- Rogers, S. M., Matheson, T., Despland, E., Dodgson, T., Burrows, M. and Simpson, S. J.** (2003). Mechanosensory-induced behavioural gregarization in the desert locust *Schistocerca gregaria*. *J. Exp. Biol.* **206**, 3991–4002.
- Rosenthal, S. B., Twomey, C. R., Hartnett, A. T., Wu, H. S. and Couzin, I. D.** (2015). Revealing the hidden networks of interaction in mobile animal groups allows prediction of complex behavioral contagion. *Proc. Natl. Acad. Sci. USA* **112**, 4690–4695.

- Rubenstein, M., Cornejo, A. and Nagpal, R. (2014). Programmable self-assembly in a thousand-robot swarm. *Science* **345**, 795–799.
- Savall, J., Ho, E. T. W., Huang, C., Maxey, J. R. and Schnitzer, M. J. (2015). Dexterous robotic manipulation of alert adult *Drosophila* for high-content experimentation. *Nat. Methods* **12**, 657–660.
- Schaefer, P. L., Varuni Kondagunta, G. and Ritzmann, R. E. (1994). Motion analysis of escape movements evoked by tactile stimulation in the cockroach *Periplaneta americana*. *J. Exp. Biol.* **190**, 287–294.
- Schneider, J. and Levine, J. D. (2014). Automated identification of social interaction criteria in *Drosophila melanogaster*. *Biol. Lett.* **10**, 20140749.
- Schneider, J., Atallah, J. and Levine, J. D. (2012a). One, two, and many—a perspective on what groups of *Drosophila melanogaster* can tell us about social dynamics. *Adv. Genet.* **77**, 59–78.
- Schneider, J., Dickinson, M. H. and Levine, J. D. (2012b). Social structures depend on innate determinants and chemosensory processing in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **109**, 17174–17179.
- Schneider, J., Atallah, J. and Levine, J. D. (2016). Social structure and indirect genetic effects: genetics of social behaviour. *Biol. Rev. Camb. Philos. Soc.* **1–12**.
- Shorrock, B. (1972). *Drosophila*. Oxford: Ginn & Company.
- Silverberg, J. L., Bierbaum, M., Sethna, J. P. and Cohen, I. (2013). Collective motion of humans in mosh and circle pits at heavy metal concerts. *Phys. Rev. Lett.* **110**, 228701.
- Simon, J. C. and Dickinson, M. H. (2010). A new chamber for studying the behavior of *Drosophila*. *PLoS ONE* **5**, e8793.
- Simon, A. F., Chou, M.-T., Salazar, E. D., Nicholson, T., Saini, N., Metchev, S. and Krantz, D. E. (2011). A simple assay to study social behavior in *Drosophila*: measurement of social space within a group. *Genes Brain Behav.* **11**, 243–252.
- Simpson, S. J., Despland, E., Hägele, B. F. and Dodgson, T. (2001). Gregarious behavior in desert locusts is evoked by touching their back legs. *Proc. Natl. Acad. Sci. USA* **98**, 3895–3897.
- Sokolowski, M. B. (2010). Social interactions in “simple” model systems. *Neuron* **65**, 780–794.
- Straw, A. D., Branson, K., Neumann, T. R. and Dickinson, M. H. (2011). Multi-camera real-time three-dimensional tracking of multiple flying animals. *J. R. Soc. Interface* **8**, 395.
- Suh, G. S. B., Wong, A. M., Hergarden, A. C., Wang, J. W., Simon, A. F., Benzer, S., Axel, R. and Anderson, D. J. (2004). A single population of olfactory sensory neurons mediates an innate avoidance behaviour in *Drosophila*. *Nature* **431**, 854–859.
- Sumpter, D. J. T. (2006). The principles of collective animal behaviour. *Philos. Trans. R. Soc. B Biol. Sci.* **361**, 5–22.
- Turner, R. H. and Killian, L. M. (1957). *Collective Behavior*. Upper Saddle River, NJ: Prentice Hall.
- Vedel, S., Tay, S., Johnston, D. M., Bruus, H. and Quake, S. R. (2013). Migration of cells in a social context. *Proc. Natl. Acad. Sci. USA* **110**, 129–134.
- Vijendravarma, R. K., Narasimha, S. and Kawecki, T. J. (2013). Predatory cannibalism in *Drosophila melanogaster* larvae. *Nat. Commun.* **4**, 1789–1788.
- Walter, W. G. (1950). An electro-mechanical animal. *Dialectica* **4**, 206–213.
- Ward, A. J. W., Herbert-Read, J. E., Sumpter, D. J. T. and Krause, J. (2011). Fast and accurate decisions through collective vigilance in fish shoals. *Proc. Natl. Acad. Sci. USA* **108**, 2312–2315.
- Williams, R. and Lusseau, D. (2006). A killer whale social network is vulnerable to targeted removals. *Biol. Lett.* **2**, 497–500.
- Wright, A. V., Nuñez, J. K. and Doudna, J. A. (2016). Biology and applications of CRISPR systems: harnessing nature’s toolbox for genome engineering. *Cell* **164**, 29–44.
- Wu, F., Huberman, B. A., Adamic, L. A. and Tyler, J. R. (2004). Information flow in social groups. *Physica A* **337**, 327–335.
- Wyatt, C., Bartoszek, E. M. and Yaksi, E. (2015). Methods for studying the zebrafish brain: past, present and future. *Eur. J. Neurosci.* **42**, 1746–1763.
- Yamamoto, D. and Koganezawa, M. (2013). Genes and circuits of courtship behaviour in *Drosophila* males. *Nat. Rev. Neurosci.* **14**, 681–692.
- Yan, Z., Zhang, W., He, Y., Gorczyca, D., Xiang, Y., Cheng, L. E., Meltzer, S., Jan, L. Y. and Jan, Y. N. (2012). *Drosophila* NOMPC is a mechanotransduction channel subunit for gentle-touch sensation. *Nature* **493**, 221–225.
- Yuan, J., Raizen, D. M. and Bau, H. H. (2014). Gait synchronization in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **111**, 6865–6870.
- Zabala, F., Polidoro, P., Robie, A., Branson, K. M., Perona, P. and Dickinson, M. H. (2012). A simple strategy for detecting moving objects during locomotion revealed by animal-robot interactions. *Curr. Biol.* **22**, 1344–1350.
- Zeltner, E., Klein, T. and Huber-Eicher, B. (2000). Is there social transmission of feather pecking in groups of laying hen chicks? *Anim. Behav.* **60**, 211–216.
- Zwarts, L., Versteven, M. and Callaerts, P. (2012). Genetics and neurobiology of aggression in *Drosophila*. *Fly* **6**, 35–48.