Debugging collective digging in *Drosophila melanogaster* using an agent-based model

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Abstract

Being a member of a group entails a range of possible costs and benefits; observing when and with whom groups form can help identify how these are weighted. Clustering behaviour in fruit fly (*Drosophila melanogaster*) third-instar larvae has been shown to provide individuals that cluster with improved foraging opportunities on otherwise inaccessible food. To isolate how environmental and genetic factors affect who clusters and when, we created an on-lattice agent-based model and systematically explored the effects of several variables, such as population density, resource distribution, and the phenotypic composition of the group. Our simple model reproduces most of the key features of larval clustering and demonstrates drastic differences in collective behaviour and fitness in response to changes in environmental variables, in ways that align with the limited empirical data available. This model can improve our understanding of this complex social behaviour and make testable predictions about the behavioural and ecological mechanisms of larval clustering.

Keywords: agent-based model, clustering, collective foraging, *Drosophila melanogaster*, larvae, social behaviour

Introduction

Individuals of a wide range of species, from ants to humans, spend a large proportion of their lives in social groups (Krause & Ruxton, 2002). The requirements for animals to be considered part of a social group are that the individuals are located closely together in space, stay together for some amount of time, and that they aggregate for social reasons (i.e., not because they were all independently attracted to some external stimulus; Burg et al., 2013; Navarro & del Solar, 1975; Pitcher, 1983; Simon et al., 2012). There are many potential benefits to joining a group, including increased access to resources, improved mating opportunities, and protection from predators (Giraldeau, 1984; Krakauer, 1995; Nagy et al., 2020; Pike et al., 2008; Ramdya et al., 2017). There are also often costs to being in a group, such as being more visible to potential predators, a heightened risk of within-group aggression, and faster depletion of limited resources (Ioannou et al., 2019; Krebs & Davies, 1996). For example, as the number of individuals in a group increases, so does the degree of competition over finite resources such as food and mates (Majolo et al., 2008).

The fruit fly, *Drosophila melanogaster*, is one of the most commonly used model organisms in genetic and developmental studies, and has also been the subject of extensive research on social behaviour (Chen & Sokolowski, 2022; Durisko & Dukas, 2013; Durisko et al., 2014; Lone & Sharma, 2011; Ramdya et al., 2014; Rooke et al., 2020; Siva-Jothy & Vale, 2019; Ueda & Kidokoro, 2002). Adult fruit flies frequently aggregate during foraging, use social information when choosing egg-laying sites, and use appendage-touching for communication (Navarro & del Solar, 1975; Dombrovski et al., 2017; Durisko et al., 2014; Lihoreau et al., 2016; Ramdya et al., 2017; Sarin & Dukas, 2009; Simon et al., 2012). Fruit fly larvae have also been observed engaging in cooperative foraging behaviours during a pivotal developmental stage – the

third instar – immediately prior to pupating (Dombrovski et al., 2017; Durisko et al., 2014; Khodaei & Long, 2019). On hard surfaces and/or under crowded conditions, fly larvae will aggregate with conspecifics and the cluster will start digging down into the substrate, with individuals synchronizing their movements (Dombrovski et al., 2017; Durisko et al., 2014). Clusters generally break up once their "mines" become so deep that they risk collapsing and losing their air supply (Dombrovski et al., 2017).

Larval clusters occur in crowded environments and/or in environments with poor food quality (Dombrovski et al., 2020; Durisko et al., 2014; Khodaei et al., 2020; Shoot et al., 2024). They tend to form on hard food or where the surface of the food has already been partially dug up (Durisko et al., 2014), as clusters allow participating larvae access to deeper, undisturbed resources. We have recently shown that larvae form deeper clusters when food quality is low or if low quality food is layered over higher-quality food (Shoot et al., 2024). Although individuals can attempt solo burrowing, this is much more dangerous than digging in a group, as the likelihood of a mine collapse increases (Dombrovski et al., 2017). Fruit fly larvae access more food when digging as a group, due to the synchronization of their digging actions (similar to when one is paddling a canoe and synchronizing strokes allows the canoe to travel more smoothly and faster through the water), allowing larger and deeper mines to be excavated (Dombrovski et al., 2017). Larvae in clusters can also feed for longer, as the mines dug by groups are larger and allow for better air exchange than mines dug by individuals (Dombrovski et al., 2017). The increase in food intake improves the chances that larvae that engage in clustering will successfully pupate (Khodaei & Long, 2019). Larvae that survive to pupate gain another fitness benefit: adult flies that clustered as larvae have larger wings and are heavier as adults (Dombrovski et al., 2020; Khodaei & Long, 2019).

There are, however, potential costs to clustering. Even though clustering increases access to food, this food is more liquefied and partially digested and is of lower quality than undisturbed food (Borash et al., 1998; Khodaei & Long, 2019), which delays the development of larvae that cluster (Dombrovski et al., 2020). In a natural context, this would also extend the time larvae are at risk of predation and/or parasitism, though larvae may be partially shielded from these risks when in the mines (Durisko et al., 2014). Additionally, initializing a cluster requires an investment of physical exertion (synchronously digging the mine) before the activity yields any rewards, and necessitates a sharing of resources among the participants. It has therefore been suggested that larvae preferentially cluster with kin, offsetting some of these costs with indirect fitness gained by assisting kin (Khodaei & Long, 2019). This selectivity may also improve the coordination of movement in collective digging, as related larvae are more likely to have similar rhythm phenotypes (Khodaei & Long, 2019). Thus, the clustering behaviour displayed by third instar *Drosophila* larvae provides a rich model system for the study of invertebrate social behaviour.

Clustering behaviour is further complicated by the two distinct foraging phenotypes present in fruit fly larvae: rovers and sitters (Anreiter & Sokolowski, 2019; Reaume & Sokolowski, 2009). As these names suggest, rovers travel over a larger area during foraging compared to the more sedentary sitters (Osborne et al., 1997; Reaume & Sokolowski, 2009; Ueno and Takahashi, 2020). The genetic difference between rovers and sitters is due to sequence differences at the *for (foraging)* locus that encodes a cGMP-dependent protein kinase (Osborne et al., 1997), which has been shown to impact a wide range of behaviours in a range of taxa (see Table 1 in Reaume & Sokolowski, 2009). Wild fruit fly populations consist of about 70% rovers

(*rover* is the dominant allele; de Belle & Sokolowski, 1989; Sokolowski, 1980; Sokolowski, et al., 1986).

Although there are no studies (that we are aware of) that have empirically explored the effects of the different alleles at the *for* loci on the expression of larval clustering, we have good reason to expect that these different larval foraging phenotype will also be reflected in their clustering dynamics. For example, the different alleles at the *for* loci are not expressed on non-nutritive substrates (Reaume & Sokolowski, 2009). In standard environments, rovers require less food than sitters due to their increased absorption of glucose and consequently develop faster (Kaun et al., 2007). Under conditions of insufficient food, rovers eat equivalent amounts of food to sitters, but their increased glucose absorption means that they survive better under these harsher conditions (Kaun et al., 2007).

At high population densities, populations contain more rovers, whereas sitters are predominantly found at low population densities (Sokolowski et al., 1997). Recent research has shown differences in sociability between rovers and sitters, with sitters spending significantly more time interacting and reciprocating conspecific interactions, whereas rovers have a significantly higher interaction rate at shorter intervals (although this may be due to rovers moving more; see Alwash et al., 2021). Rovers learn faster and retain memories better in the short term, whereas sitters have slower memory acquisition with a shorter retention in the short term, but have better long-term memory (Kaun et al., 2007b; Mery et al., 2007). As there are only two phenotypes, and their differences are well-documented, this system lends itself well to creating a simple theoretical model of the effect of individual variation on group success.

Here, to better understand the behavioural, genetic, and cognitive mechanisms of clustering, we present an agent-based model of the phenomenon that captures how clustering

emerges from the interactions between individuals, and the effects of individual social and physical abilities on the expression of the behavioural phenotype. We use our model to explore the underlying mechanisms of clustering behaviour, and how individual differences or environmental heterogeneity affect the success of collective foraging in fruit fly larvae. We first address the effects of environmental factors on the effectiveness and prevalence of clustering (Model 1), and then additionally consider the effects of phenotypic variation (Model 2).

Our model is designed to capture the key known aspects of fruit fly larval clustering. First, our agents can obtain food by foraging alone at the surface, not only by clustering, although they may obtain less food in this way, reflecting the higher efficiency of collective foraging (Dombrovski et al., 2017). Foraging at the surface decreases the amount of food available there, mimicking the lower quality of dug-up substrates. Second, initiating a cluster in the model requires an initial investment that yields little reward (Khodaei & Long, 2019; though we also explore the effects of relaxing this assumption). Finally, environmental resources in the model are layered such that deeper mines access better food (Shoot et al., 2024), and we also explore the effects of varying the horizontal distribution of resources.

Methods

Environment

Our model is a discrete-time agent-based model, with synchronous updating (Granovskiy, 2012). It is an on-lattice model, which means that the space on which agents behave is divided into cells, rather than being continuous. Cells can hold more than one agent, and each agent must occupy a specific cell at each point in time. All simulations take place on a 50 x 50 cell arena with periodic boundaries.

Each cell of the arena is characterized by the amount of surface food available there – accessible to agents foraging alone – and by how much clustering has already taken place there. All cells start each simulation run with no digging having occurred and with the maximal amount of food available at the surface (which is uniform across all cells and = 0.5). If an agent forages alone in a cell, the amount of food available at the surface of that cell decreases by a fixed amount per timestep (which = 0.01 food in our simulations), until it reaches 0. Food does not replenish.

The amount of food agents can gain from a given cell by clustering there depends on the depth of the mine they have created (simulating, for example, having to penetrate the initial agar in a vial of food; see also Shoot et al., 2024). We track the amount of clustering (how many agents for how many time-steps) that has occurred in each cell. Each cell progresses through three phases (or layers; Φ) as it is dug deeper: the top phase provides 0.3 units of food per clustering agent per time-step; the second phase, 0.8; and the third phase returns no food – the cluster is too deep and cannot be maintained. Clustering is immediately more rewarding than foraging at the surface (the initial phase of clustering yields 0.3 units per timestep compared to surface foraging, which yields 0.01 units per timestep). Digging through phase 1 into phase 2 requires 20 digging steps (each agent clustering for one timestep accounts for one digging step), and 80 more steps are required to dig beyond phase 2 into phase 3. These values were selected arbitrarily, as we are aware of no existing relevant empirical data.

Model 1 Agents

Agents are characterized by their current position, food reserve level, and behavioural state. They are spawned onto the arena at random locations at the start of each simulation run, and all begin with a food reserve of 0.2 (to avoid premature death). There are three states agents

can occupy at each time-step: moving, foraging (at the surface), or clustering (with others, below the surface). Figure 1 shows the decision tree that determines an agent's chosen state (see Table S1 for the state transition matrix). Agents that are not alone in their cell must cluster, and they cannot cluster if they are alone (though agents could, in theory, dig down into the substrate alone, they would not thereby obtain the benefits of clustering and this strategy would be less advantageous than foraging at the surface [Khodaei & Long, 2019]; we therefore chose to exclude this behavior from the model). Clusters disband when the cell reaches phase 3 (the cluster is too deep to maintain or the resources have been depleted), and all agents must then move. Agents can also leave clusters before this time, which is more likely in the less-rewarding phase 1 than in phase 2. Agents that are not clustering choose to either forage or move depending on their current food reserves (S) and the current rate of intake of the cell they are occupying (q), employing a form of marginal value theorem (Charnov, 1976). The probability of transitioning to foraging from moving (or continuing to forage) is given by $Ps = [(M\text{-}2q)\ (S\text{-}1)]/10M + (q/M)$, where M = the maximal food intake rate (= 0.5). The probability of transitioning to moving from foraging (or continuing to move) is $1 - P_S$. The function is illustrated in Figure S2.

At each time-step, agents incur a fixed metabolic cost of 0.05. Moving incurs an additional cost of 0.05. Agents can move at a fixed speed of 1 cell (in any direction, including diagonals) per time-step. Agents whose food reserves fall to 0 die and are removed from the simulation.

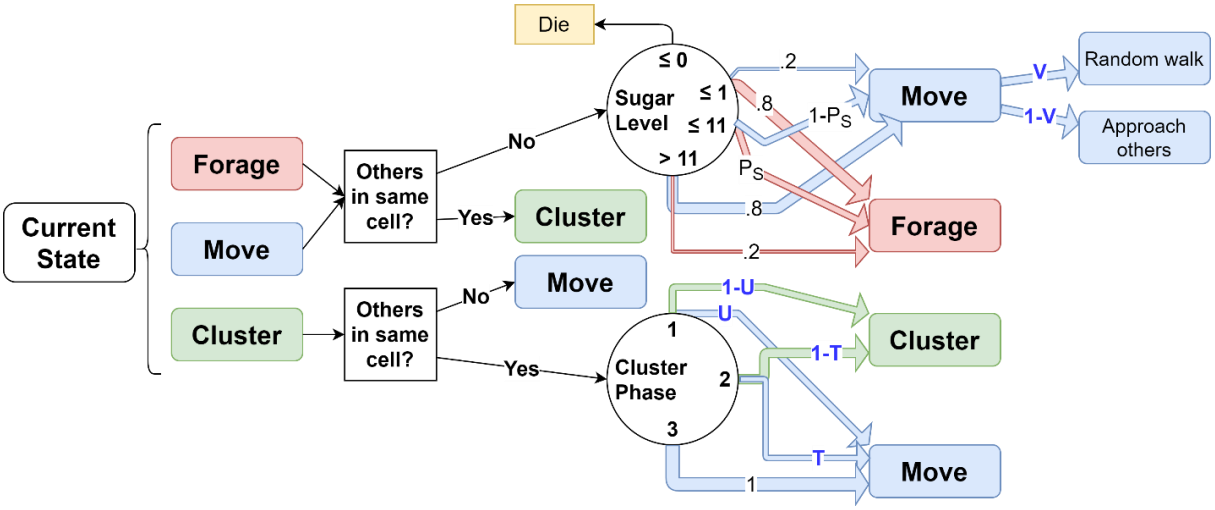

Figure 1. Agent decision tree for both models. Differences between the models are indicated in dark blue. If the focal larva is either foraging or moving, and there are other larvae in the same cell, it clusters (top left). The likelihood of continuing to cluster then depends on what phase the current mine is in (bottom circle): in Model 1, T = 0.1 and U = 0.25 (Table S1); in Model 2, T = 0.35 and U = 0.5 for rovers (sitters behave like agents in Model 1). If there are no other agents in the same cell, the probability of moving or foraging is contingent on current food reserves (top circle). If the food level is between 1 and 11, the probability of moving or foraging is dictated by *Ps* (illustrated in Figure S2). When moving (top right), V = 0.5 in Model 1, making agents equally likely to move towards a nearby individual or randomly; in Model 2, V = 0.8 for rovers (agents classified as sitters behave like agents in Model 1).

If an agent chooses to move, it surveys all the surrounding cells that are within 5 cells of its location (the limit of its visual range) and identifies all other agents within that area. Agents will either move towards their nearest neighbour or in a random direction, with probability 0.5. If there are no other agents within their visual range, they always move in a randomly selected direction.

Model 2 Agents

For Model 2, rover and sitter phenotypes were added to the Model 1 agents. The rover population was initially set at 70% of the total population (Sokolowski, 1980). Sitters behave exactly like the agents in the Model 1 simulations. Rovers behave differently in just 2 ways:

1. When clustering, rovers are more likely than sitters to leave the cluster before it reaches phase 3. Rovers' probability of transitioning from clustering to moving is 0.5 in a cell's phase 1 (it is 0.25 for sitters) and 0.35 in phase 2 (it is 0.1 for sitters), simulating rovers' greater propensity to move.

2. When moving, rovers move towards their nearest neighbour with probability 0.2 (it is 0.5 for sitters), and in a random direction otherwise (See Figure 1). This simulates sitters' higher sociability (Alwash et al., 2021).

Simulations were run in Python (v.3.8) and 100 replications were run for each set of variable values. The dependent variables we measured were: mean final food amount, proportion of time spent clustering, cluster size (number of agents), and cluster duration. These variables were chosen to describe cluster attributes and assess the fitness benefits of clustering. Although we explored the effects of varying many model parameters, in the main text we report only the key parameters that were found to have consequential effects on the dependent variables. Effects of other parameters that we varied are presented in the SI. We varied the number (density) of agents, how rewarding each phase of clustering was (the vertical distribution of resources), and the distribution of food available for individual foraging at the surface (the horizontal distribution of resources). In the SI, we further investigate the effects of varying the visual range of agents, their fixed metabolic cost, and their movement speed. We varied one parameter at a time, keeping the others at baseline values. Table 1 shows the range of values taken on by each parameter, for results discussed in the main text; baseline values are bolded. Other parameter values are given in Table S2.

Parameter	Symbol	Values simulated
Model 1		
Population size (density)	N	25, 50, **100**, 150, 200, 250, 300, 350
Phase rewards {phase 1, phase 2}	$\boldsymbol{\theta}$	{**0.3,0.8**}, {0.8, 0.8}, {0.8, 0.3}, {0.1, 0.8}
Resource distribution	σ	**Uniform**, Random, Peaked (von Mises)
Model 2		
Population size (density)	N	25, 50, **100**, 150, 200, 250, 300, 350
Phase rewards {phase 1, phase 2}	$\boldsymbol{\theta}$	{**0.3,0.8**}, {0.8, 0.8}, {0.8, 0.3}, {0.1,0.8}
Population composition R:S	$\boldsymbol{\rho}$	**70:30**, 30:70, 50:50

Table 1: Model parameter symbols and values. 100 simulations were run at each set of values with a set 100 timesteps per simulation run. Bolded values are the baseline and were held constant while varying other parameters. Only one parameter was varied at a time.

Analysis

All analyses were conducted in R (v.3.6.1). One-way ANOVAs were used to examine the effects of each independent variable. Since our data were not homogenous, and some data were proportional, we applied a Welch correction to all analyses. We report effect sizes for all analyses (η^2) and post-hoc tests with a Bonferroni correction for multiple comparisons. Raw data (simulation outputs and summaries), code for running the model, and analysis scripts are available in our OSF data repository (https://osf.io/7agcz/?view_only=d358a86036bc4821b7f6a0419fdd9992).

The same analyses were conducted for both models, with the exception that, where possible (for cluster duration and final food amounts), a repeated-measures ANOVA was used to allow the inclusion of phenotype (rover or sitter) in the analyses for Model 2; phenotype was

treated as a within-subjects effect since we analyzed averaged data from each run of the simulation.

Results

Model 1

We present results separately for each independent variable. For each set of conditions, we measured: mean *cluster duration* – how many time-steps a cluster persisted for before one or more members moved away; mean *cluster size* – the number of individuals participating in each cluster; *final food* – the mean food stores of each larva at the end of the simulation (a good proxy for fitness); and the mean *proportion of time* larvae spent clustering.

Population Density

We first varied the number of agents spawned onto the arena, N, from 25 to 350 (see Table 1). As the size of the arena is fixed (2500 cells), this is the same as varying the density of larvae from 0.01 to 0.14 larvae / cell.

The mean duration of cluster persistence initially increased with N, from N = 25 to N = 50, and then decreased for Ns > 100 (Figure 2A; ANOVA: $F(7, 348.7) = 432.4$, $P < 0.001$, $\eta^2 = 0.44$; post-hocs showed all $P < 0.001$ except N = 25 vs. 150, 50 vs. 100 [both P = 1], 50 vs. 150 [P = 0.096], 100 vs. 150 [P = 0.01], and 300 vs. 250 and 350 [both P > 0.01]). The mean size of clusters also increased with N (Figure 2A; ANOVA: $F(7, 369.3) = 2217.5$, $P < 0.001$, $\eta^2 = 0.83$; post-hoc tests showed $P < 0.001$ for all comparisons except N = 300 vs. 350 [P = 0.002]). Final food levels increased with N up to 150 and decreased thereafter (Figure 2B; ANOVA: $F(7, 357.8) = 241.4$, $P < 0.001$, $\eta^2 = 0.46$; post-hoc tests showed all $P < 0.001$, except N = 50 vs. 250 [P = 0.32], 100 vs. 150 [P = 0.26], 150 vs. 200 [P = 0.11], 50 vs. 300, and 100 vs. 200 [both P =

1]), and the proportion of time that agents spent clustering increased until N = 100 and then stabilized (Figure 2C; ANOVA: $F(7, 390.2) = 717.0$, $P < 0.001$, $\eta^2 = 0.36$; post-hoc tests showed all $P < 0.001$ when one $N \leq 100$, except N = 50 vs. 100 [P = 1], all other P = 1).

These results suggest that there is an optimal intermediate density that increases the fitness of the agents, by promoting increased stability and size of clusters: at low densities, clusters are rare and therefore harder to locate and join, leading to less time spent clustering and correspondingly lower success; at very high densities, larger clusters deplete many locations, requiring reorganization in a new, undepleted, location (this also causes the slight decrease in cluster duration at high densities; Figure 2A). As further evidence for this explanation, we note that the proportion of cells that had any clustering occurring in them increased with N (Figure S1; ANOVA: $F(7, 338.8) = 2191.0$, $P < 0.001$, $\eta^2 = 0.96$; post-hoc tests showed $P < 0.001$ for all comparisons) as did the proportion of those cells that reached phase 3 – when the location is depleted and no longer provides any food (Figure S1; ANOVA: $F(7, 326.9) = 9021.9$, $P < 0.001$, $\eta^2 = 0.98$; post-hoc tests showed $P < 0.001$ for all comparisons).

Phase Rewards

We next explored the effect of modulating how much food was obtained at the two topmost phases of clustering in each cell (the third phase, in all simulations, yields no food). In our baseline simulations, the topmost phase (phase 1) yields little food (0.3 units per timestep per agent) compared to the middle phase (phase 2, which gives 0.8 units per timestep per agent), simulating, for example, a typical nutritionally stratified environment that occurs in stock vials where the topmost phase becomes depleted through larval foraging while the lower layers remain relatively intact, since clusters are normally found on harder or partially dug up substrate (Durisko et al., 2014; Shoot et al., 2024). In the baseline condition, phase 1 rewards are also less

than the 0.5 units that can be obtained by solitary foraging at the surface in untouched cells. We tested the effects of making this imbalance even more extreme (by reducing phase 1 rewards even further: phase 1 = 0.1, phase 2 = 0.8), evening out the two phases (phase 1 = phase 2 = 0.8), and flipping the reward structure (phase 1 = 0.8, phase 2 = 0.3). We report phase reward levels as {phase 1, phase2} (e.g., the baseline condition is {0.3, 0.8}).

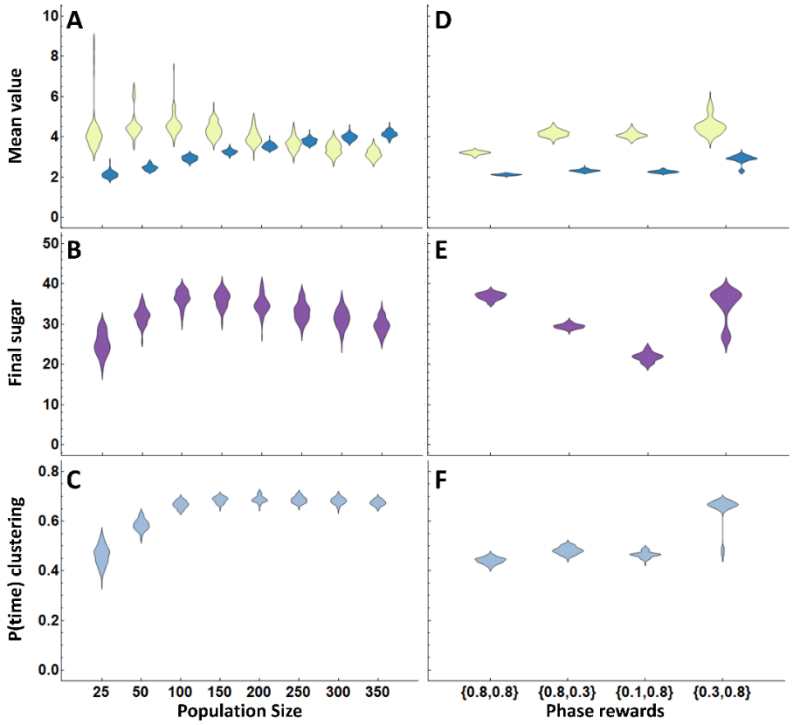

Figure 2. Consequences of varying agent density and phase reward structure. Violin plots of (**A**, **D**) mean cluster duration (yellow) and size (blue); (**B**, **E**) final food; and (**C**, **F**) proportion of time spent clustering, as a function of total population size (**A** - **C**) or the rewards obtained at each phase of clustering (**D** - **F**).

Changing phase rewards affected how long clusters survived (Figure 2D; ANOVA: $F(3,200.3) = 1520.2$, $P < 0.001$, $\eta^2 = 0.80$; post-hoc tests showed $P < 0.001$ for all comparisons except {0.1, 0.8} vs. {0.8, 0.3} [$P = 0.29$]), as well as their size (Figure 2D; ANOVA: $F(3,208.1) = 1653.7$, $P < 0.001$, $\eta^2 = 0.95$; post-hoc tests showed $P < 0.001$ for all comparisons). Final food levels were also affected (Figure 2E; ANOVA: $F(3,208) = 3984$, $P < 0.001$, $\eta^2 = 0.96$; post-hoc tests showed $P < 0.001$ for all comparisons except {0.3, 0.8} vs. {0.8, 0.8} [$P = 0.004$]), as was the proportion of time spent clustering (Figure 2F; ANOVA: $F(3,219) = 35{,}665$, $P < 0.001$, $\eta^2 = 1$; post-hoc tests showed $P < 0.001$ for all comparisons).

These results suggest that our simple agents can assess the differences in food quality between the layers to maximize resource acquisition (indirectly; agents can only detect their current rate of intake). It is also worth noting that agents' decisions to leave clusters are not sensitive to the rate of reward (the likelihood of leaving the cluster is a fixed probability, dependent only on cluster phase). The only part of agents' decision-making process that is sensitive to food intake levels is whether to forage or move when alone, and only if food levels are between 1 and 11 (see Figure 1). How does this mediate all the differences we find between conditions when varying the clustering reward structure?

Interestingly, either decreasing or increasing the rewards available in the first phase of clustering both led to a decrease in time spent clustering (Figure 2F). Figure S2 shows the function P_S, that determines an agent's choice to forage or move. When food reserves are high, agents are more likely to move when the foraging rate is high – in search of opportunities to cluster; when they have low food reserves, however, agents are more likely to stay and forage at the surface when the reward rate for that behaviour is higher. Thus, when phase 1 of clustering is made even less rewarding than the baseline condition, in the $\{0.1, 0.8\}$ condition, this increase in initial costs causes agents that leave the cluster before it reaches phase 2 to have lower food reserves, increasing the chance that they will forage at the surface rather than moving, and thus reducing their chances of locating another cluster to join. This explains why agents in this condition spend less time clustering (Figure 2F), and have shorter cluster durations and smaller cluster sizes. Since clustering is still a good strategy in this environment (once the cluster reaches phase 2), agents in this condition that are discouraged from clustering end up with lower food levels (Figure 2E).

In the {0.8, 0.3} condition, in which phase 1 is very rewarding (more so than foraging at the surface), agents that leave the cluster during this phase likely have food reserves that exceed 11 units, at which point the decision function is no longer invoked and the probability of foraging is 0.8. These agents therefore forage more than those in the baseline condition (who have a less rewarding cluster phase 1), and move less, becoming less likely to find future clusters and reducing their total clustering time. Clusters that do reach phase 2 in this condition are less rewarded, resulting in lower overall final food levels (Figure 2E). A similar effect occurs in the {0.8, 0.8} condition, leading to little time spent clustering compared to the baseline condition (Figure 2F). However, clustering in phase 2 in this condition is also rewarding (as rewarding as in the baseline condition), leading to overall high final food levels (Figure 2E).

Resource distribution

Finally, we manipulated the distribution of resources in the arena. In our baseline condition, the initial food level available for foraging at the surface was uniform across all cells, but natural food distributions will often be patchy or otherwise uneven. We therefore tested the effects of a randomized distribution of foods and a peaked (von Mises) distribution. All three distributions (uniform, random, and peaked) had the same mean initial value (0.5).

We found no overall effect of resource distribution on cluster duration (Figure S3. ANOVA: $F(2, 197.9) = 4.27$, $P = 0.015$, $\eta^2 = 0.03$), though there was a marginal difference between the Random (mean = 4.10 ± 0.19 SD) and Peaked conditions (4.17 ± 0.19; post-hoc comparison, $P = 0.013$; both other comparisons, $P > 0.1$). Resource distribution did have an effect on cluster size (Uniform: 2.30 ± 0.06; Random: 2.26 ± 0.06; Peaked: 2.30 ± 0.06; ANOVA: $F(2,197.8) = 16.23$, $P < 0.001$, $\eta^2 = 0.10$), with the Random different from both the Uniform and Peaked distributions (both $P < 0.001$; Peaked vs. Uniform, $P = 1$). Final food

amounts also varied with resource distribution (ANOVA: $F(2,198) = 8.35$, $P < 0.001$, $\eta^2 = 0.05$; post-hocs showed that the Random distribution was different from both Uniform [$P = 0.002$] and Peaked [$P < 0.001$]; Peaked and Uniform were not different from each other [$P = 1$]). Proportion of time spent clustering was weakly affected by resource distribution (Uniform: 0.48 ± 0.02; Random: 0.47 ± 0.02; Peaked: 0.48 ± 0.02; ANOVA: $F(2,197.9) = 5.32$, $P = 0.006$, $\eta^2 = 0.04$; post-hoc tests: Uniform vs. Random, $P = 0.025$; Uniform vs. Peaked, $P = 1$; Peaked vs. Random, $P = 0.008$).

These results suggest that varying the distribution of resources, at least within the limits that we attempted here – while keeping the mean level in the arena constant – has only a limited effect on the dynamics of cluster formation and, more importantly, on the fitness consequences of behaviour. Our agents are not able to detect resource gradients, except indirectly (movement decisions can depend on the current rate of foraging in some situations, as discussed above), and it is therefore likely that the absence of effects we observed results from averaging across agents that happened to do both better and worse than in the baseline simulation (as evidenced by the increased variance in final food levels [Figure S3B] and proportion of time clustering [Figure S3C] in this condition). Real fruit fly larvae will preferentially cluster in richer areas (Shoot et al., 2024).

Model 2

We first note that, in all our simulations, sitters appear to do better (accumulate more food) than rovers (Figure 4B). This is due to sitters spending more time in clusters (Figure 4D) that last for longer (and are therefore more likely to reach the deeper, more rewarding phase; Figure 4A), as rovers are more likely to leave clusters and, when moving, less likely to approach others and form new clusters. In reality, there are several other differences between sitters and

rovers (such as in glucose absorption; Anreiter & Sokolowski, 2019) which our model does not include – as we are focused on clustering behaviour – and which likely serve to equalize the fitness of the two phenotypes under most conditions. In our model, rovers are under-represented in clusters (Figure S4), due to these same rules, and clusters with a higher proportion of rovers do not last as long and tend to be smaller (Figure S5). We are not aware of any empirical data on the relative proportions of rovers and sitters in foraging clusters, but future work could test the model's predictions on this, as well as modeling the effects of variance in glucose absorption.

Population density

As in Model 1, we first varied the number of agents spawned onto the arena, N, from 25 to 350 (see Table 1). As the size of the arena is fixed (50 x 50 cells), this is the same as varying the density of larvae from 0.01 to 0.14 larvae / cell.

There were significant effects of both phenotype (Figure 3A; $F(1,792) = 24943.12$, $P < 0.001$, $\eta^2 = 0.871$) and density ($F(7,792) = 160.35$, $P < 0.001$, $\eta^2 = 0.045$; post-hocs showed all $P < 0.001$ except N = 200 vs. 250 [$P = 0.230$] and 250 vs. 300, and 300 vs. 350 [both $P = 0.029$]) on the duration of clustering, and an interaction between phenotype and density ($F(7,792) = 98.565$, $P < 0.001$, $\eta^2 = 0.024$). The mean size of clusters significantly increased as population density increased (Figure 3C; $F(7,792) = 365.8$, $P < 0.001$, $\eta^2 = 0.76$; post-hoc tests showed $P < 0.001$ for all comparisons except for 200 vs. 250 [$P = 0.233$] and 300 vs. 350 [$P = 0.003$]). Final food levels also showed significant effects of phenotype (Figure 3B; $F(1,792) = 22328.649$, $P < 0.001$, $\eta^2 = 0.630$), density ($F(7,792) = 885.550$, $P < 0.001$, $\eta^2 = 0.251$; post-hoc tests showed all $P < 0.001$), and an interaction between phenotype and density ($F(7,792) = 324.278$, $P < 0.001$, $\eta^2 = 0.064$). Proportion of time clustering was significantly affected by phenotype (Figure 3D; $F(1,693) = 36603.678$, $P < 0.001$, $\eta^2 = 0.607$), density ($F(6,693) = 2886.180$, $P < 0.001$, $\eta^2 =$

0.350; post-hoc tests showed all $P < 0.001$), and an interaction between phenotype and density ($F(6,693) = 170.901$, $P < 0.001$, $\eta^2 = 0.017$).

As in Model 1, we find that the proportion of time clustering, the size and longevity of clusters, and final food levels all increase as N increases initially. However, in Model 1, all these values either stabilized or began to decrease as N exceeded about 150, due to clusters being depleted. Here, we find that all these values continue to increase as N increases to 350 (albeit at a slower rate). This is because rovers are more likely to leave clusters early, before they become depleted, and move further away before re-forming a new cluster. This causes the clustering behaviour to spread out compared to Model 1, even at high densities (a greater proportion of the arena's cells have been clustered in; Figure S6; 2-way ANOVA with Model and density as fixed factors: effect of Model, $F(1,1584) = 311,207$, $P < .001$, $\eta^2 = 0.62$; effect of density, $F(7,1584) = 19,837$, $P < .001$, $\eta^2 = 0.28$; density * Model interaction, $F(7,1584) = 7184$, $P < .001$, $\eta^2 = 0.10$), and fewer locations to become depleted (ANOVA, excluding N=25 as no cells were depleted in that condition; effect of Model, $F(1,1386) = 74,616$, $P < .001$, $\eta^2 = 0.73$; effect of density, $F(6,1386) = 2,278$, $P < .001$, $\eta^2 = 0.13$, density * Model interaction, $F(6,1386) = 2113$, $P < .001$, $\eta^2 = 0.12$).

Phase rewards

The mean duration of clustering was affected by both phenotype (Figure 4A; $F(1,396) = 12821.325$, $P < 0.001$, $\eta^2 = 0.913$) and condition ($F(3,396) = 57.020$, $P < 0.001$, $\eta^2 = 0.014$; post-hocs showed all $P < 0.001$ except for {0.3,0.8} vs. {0.1,0.8} and {0.3,0.8} vs. {0.8,0.3} [$P = 0.627$ for both] and {0.8,0.3} vs. {0.1, 0.8} [$P = 0.811$]; the {0.8,0.8} condition was significantly lower than all others), as well as an interaction between phenotype and condition ($F(3,396) = 51.078$, $P < 0.001$, $\eta^2 = 0.011$). This is similar to Model 1,and is likely due to similar

mechanisms such as being able to assess differences in quality of food. The mean sizes of clusters were significantly different across some conditions (Figure 4C; ANOVA: $F(3,396) = 9.25$, $P < 0.001$, $\eta^2 = 0.07$; post-hoc tests showed $P < 0.001$ for all comparisons except for {0.3,0.8} vs. {0.8,0.3} [$P = 0.859$] and {0.3,0.8} vs. {0.1,0.8} [$P = 0.436$] and {0.8,0.8} vs. {{0.1,0.8} [$P = 0.049$] and {0.8,0.3} vs. {0.1,0.8} [$P = 0.101$]). Final food levels showed significant effects of phenotype (Figure 4B; $F(1,396) = 16286.196$, $P < 0.001$, $\eta^2 = 0.332$) and condition ($F(3,396) = 6736.987$, $P < 0.001$, $\eta^2 = 0.583$; post-hoc tests showed all $P < 0.001$), as well as an interaction between phenotype and condition ($F(3,396) = 1075.992$, $P < 0.001$, $\eta^2 = 0.066$). Proportion of time clustering also showed significant effects of phenotype (Figure 4D; $F(1,297) = 20863.144$, $P < 0.001$, $\eta^2 = 0.966$), condition ($F(2,297) = 13.476$, $P < 0.001$, $\eta^2 = 0.002$; post-hoc tests showed all $P < 0.001$, except for {0.8,0.8} and {0.1,0.8}), and an interaction between phenotype and condition ($F(2,297) = 6.645$, $P = 0.002$, $\eta^2 = 0.00062$).

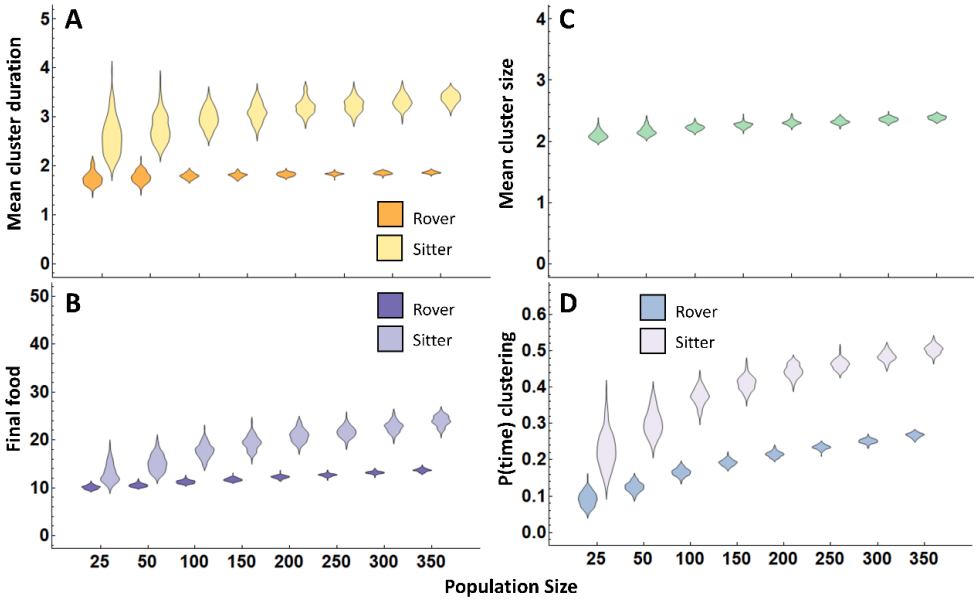

Figure 3. Effects of phenotype and population density. Violin plots for mean cluster duration (A), final food (B), mean cluster size (C), and proportion of time clustering (D), as a function of total population size. Rovers are represented by the darker colour in each panel, with the exception of mean cluster size.

These results, interestingly, do not show larvae achieving greater success in the standard model {0.3,0.8} over other conditions, as we found in Model 1. For both rovers and sitters, the

{0.8,0.8} and {0.8,0.3} conditions yielded the highest final food levels. This result is likely due to many of the clusters dissolving before reaching phase 2 (Figure S7), due to rovers' greater likelihood of leaving clusters (in Model 1, all agents are sitters). Thus, the success of clustering depends almost entirely on the reward gained in phase 1, which is likely why the {0.1,0.8} condition had the lowest final food, followed by the {0.3,0.8} condition, and then the two conditions in which phase 1 gave 0.8 food units per timestep.

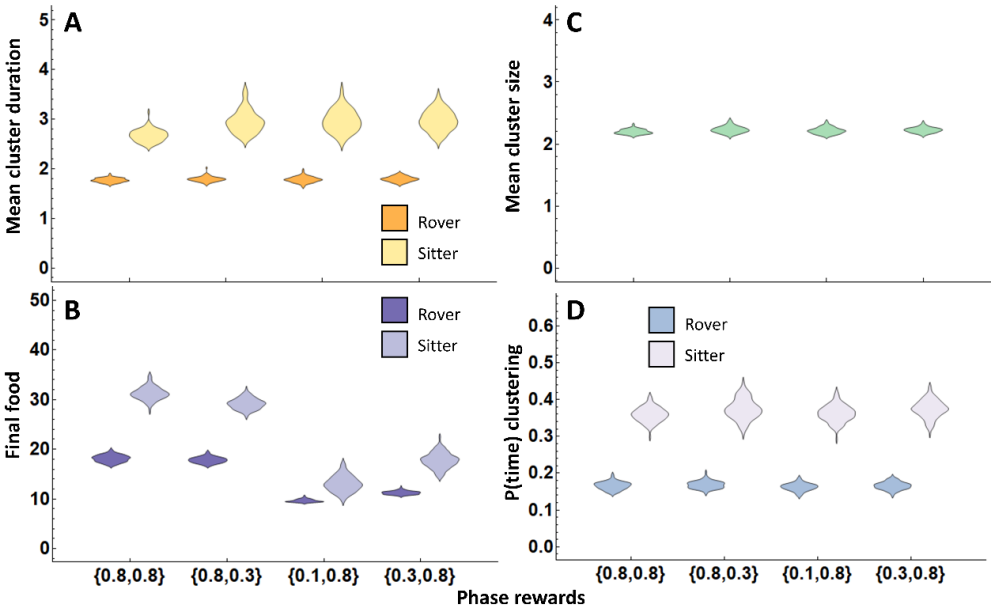

Figure 4. Effects of phenotype and phase rewards. Violin plots for mean cluster duration (A), final food (B), mean cluster size (C), and proportion of time clustering (D), as a function of phase rewards. Rovers are represented by the darker colour, apart from mean cluster size.

Population composition

The mean duration of clustering was significantly affected by both phenotype (Figure 5A; $F(1,297) = 15817.378$, $P < 0.001$, $\eta^2 = 0.891$) and composition ($F(2,297) = 370.862$, $P < 0.001$, $\eta^2 = 0.046$; post-hoc tests showed all $P < 0.001$), as well as an interaction between phenotype and composition ($F(2,297) = 246.783$, $P < 0.001$, $\eta^2 = 0.028$). The mean size of clusters significantly decreased as the proportion of rovers increased (Figure 5C; $F(2,297) = 182.83$, $P < 0.001$, $\eta^2 = 0.552$; post-hoc tests showed $P < 0.001$ for all comparisons). Final food was affected by both

phenotype (Figure 5B; $F(1,297) = 11169.050$, $P < 0.001$, $\eta^2 = 0.830$) and composition ($F(2,297) = 572.016$, $P < 0.001$, $\eta^2 = 0.082$; post-hoc tests showed all $P < 0.001$), as well as an interaction between phenotype and composition ($F(2,297) = 232.575$, $P < 0.001$, $\eta^2 = 0.035$). Proportion of time clustering was also affected by phenotype (Figure 5D; $F(1,297) = 29005.880$, $P < 0.001$, $\eta^2 = 0.949$), composition ($F(2,297) = 283.024$, $P < 0.001$, $\eta^2 = 0.025$; post-hoc tests showed all $P < 0.001$), and an interaction between phenotype and composition ($F(2,297) = 56.850$, $P < 0.001$, $\eta^2 = 0.004$).

As the proportion of sitters in the population decreased, so did all measures of their clustering and their success rate (final food). Rovers, however, were more resilient to changes in population structure, though they did not achieve the same final food amounts as sitters under any conditions. These effects are most likely caused by rovers' lower probability of clustering. The more rovers there are in the population, the less clustering takes place overall, decreasing the success rate of the sitters, who would otherwise have done better by clustering.

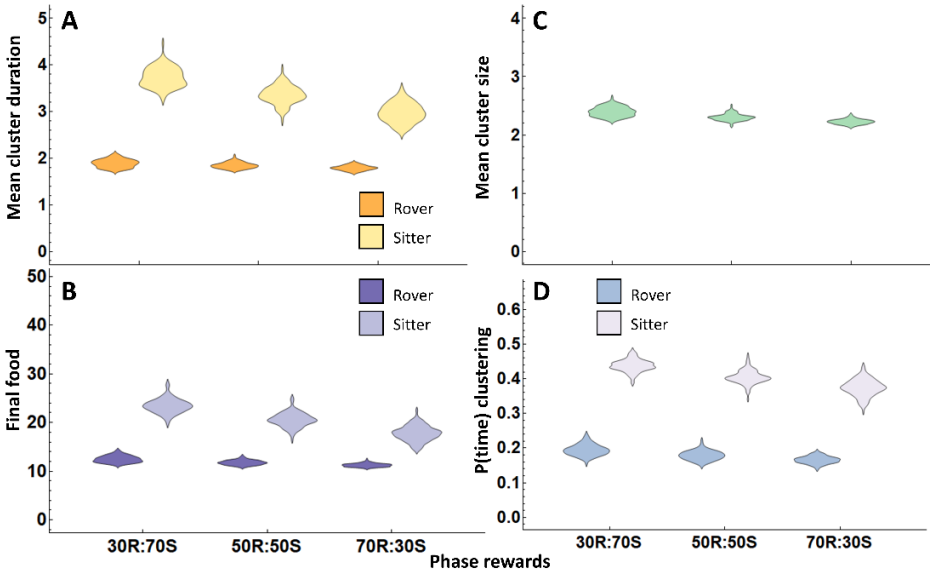

Figure 5. Effects of phenotype and population structure. Violin plots for mean cluster duration (A), final food (B), mean cluster size (C), and proportion of time clustering (D), as a function of proportions of each phenotype in the population. Rovers are represented by the darker colour, apart from mean cluster size.

Comparing across models

Agents that were sitters in Model 2 followed the same behavioural rules as the agents in Model 1. Thus, Model 1 may be considered a special case of the model for Model 2, where the population composition is 100% sitters. We compared the success rate (final food) of sitters as a function of their prevalence (Figure 6). We found a significant effect of population composition on final food levels ($F(3,246) = 152.290$, $P < 0.001$, $\eta^2 = 0.650$; post-hoc tests showed a significant difference between all groups [P <.001], except 50% vs. 30% sitters [P = .006] and 70% vs. 50% sitters [P = .927]). Additionally, as the figure shows, the presence of any rovers at all greatly increased the variance of success rates, most likely because of their disruptive effect on those clusters they are part of.

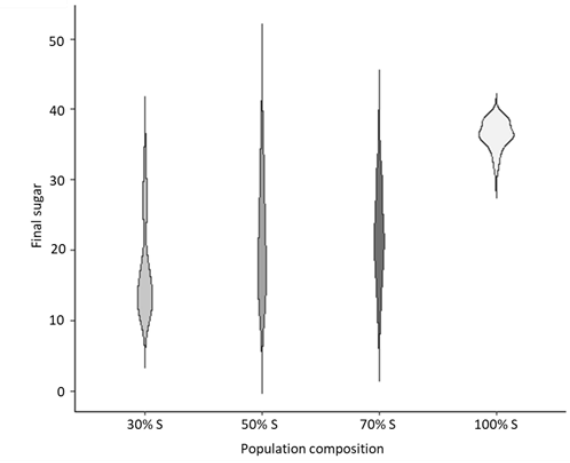

Figure 6. Effects of population structure (proportion of sitters in the population) on success rates (final food) of sitters.

Discussion

In our first model of collective foraging in fruit fly larvae, identical agents follow a set of simple rules (Figure 1) to decide whether to forage (alone), cluster (with others), or move. This model appears to reproduce the basic phenomena of larval clustering behaviour. We then varied the population density, quality of the substrate (clustering phase rewards), and resource

distribution, and show that all but the last of these affect the propensity of individuals to cluster and the size and longevity of the resulting groups.

We found that there is an optimal intermediate density of individuals that leads to the most robust clustering and highest success rates in the model (Figure 2). This finding aligns with empirical results that show that crowded conditions are necessary for cluster formation, but too much crowding can be detrimental due to increased waste production (Dombrovski et al., 2020; Durisko et al., 2014). We also found that altering the vertical distribution of resources (the relative reward values of different clustering phases) had a large effect on clustering dynamics (Figure 2), echoing the finding that clusters are generally found on poorer quality food (Khodaei et al., 2020) and that layering poor food on top of higher quality food encourages deeper clusters (Shoot et al., 2024). Changes in the horizontal distribution of resources (altering the surface foraging distributions) had smaller or no effect on clustering (or other behaviours), possibly because we kept the mean value of these resources across the arena constant.

In our second model, we investigated how the addition of phenotypic differences to our model allows it to reproduce some of the individual differences observed in wild fly populations. With only two well-described foraging phenotypes, *Drosophila melanogaster* is an ideal system in which to explore this question theoretically. We found stark differences between the two phenotypes, with sitters spending more time in larger and longer-lasting clusters, leading to higher success rates. Our model does not capture the reasons for the proportion of rovers being higher in wild populations (Sokolowski, et al., 1997), possibly because we did not alter the metabolic profile of rovers to fit empirical findings (Kaun et al., 2007).

Environmental factors like population size or reward distribution did affect the success and behaviours of agents in this model, as they did in Model 1, but in different ways. Increasing

density, for example, caused a monotonic increase in success rates for both phenotypes, without the decrease we observed at very high densities in Model 1, probably due to the rovers spreading out clustering over a larger proportion of the arena (Figure 3). Rovers' lower likelihood of remaining within clusters made most clusters dissolve before reaching the deeper, more rewarding phase 2 of clustering (Figure S7), making the reward level of the top phase the primary determinant of success rates (Figure 4). Finally, increasing the proportion of rovers in the population (Figure 5) decreased success rates, by decreasing the amount of clustering. Indeed, at no point did sitters in a mixed-phenotype population do as well as they do when alone (Figure 6).

To improve our understanding of the behavioural, genetic, and cognitive mechanisms of collective foraging in fruit fly larvae, we created an agent-based model of the clustering behaviour they display in the third instar stage. We find that our agents, who follow a set of simple fixed rules, nonetheless reproduce key aspects of clustering: clustering is most beneficial at intermediate population densities (Dombrovski et al., 2020; Durisko et al., 2014), and where the food available at the surface is of poor quality (Khodaei et al., 2020); and agents that cluster more, whether by chance or because of their phenotype, attain higher fitness (Khodaei & Long, 2019). Importantly, the model also makes some predictions that have not yet, as far as we are aware, been tested empirically: that the horizontal distribution of resources can have an effect on the amount of clustering observed and its effectiveness (Figure S3), and that the phenotypic composition of the population (such as the proportion of rovers) alters the prevalence, dynamics, and effectiveness of clustering (Figure 5), with rovers being under-represented in clusters (Figure S4). All these predictions could be tested in real fly populations (as in Shoot et al., 2024).

In addition, we made some assumptions in designing the model that could not be validated empirically (and potentially altered in the model depending on the results of such studies). For example, we assumed that, when moving, larvae are equally likely to move towards others or in a random direction. It would be interesting to test how likely larvae are to move towards others, and how this behaviour might change as a function of density or resource distributions.

In Model 2, we modified the rules followed by some agents, to simulate behavioural differences between rovers and sitters (who followed the same rules as in Model 1). Adding rovers to the population altered the dynamics of the model, by spreading out and generally reducing clustering behaviour. Though rovers consistently did less well than sitters in our model, a departure from empirical data that is likely due to factors that we did not attempt to model, our agents still adapted their behaviour to changes in environmental conditions such as density or reward distribution. The data to compare these effects to real populations is mostly lacking, though our finding that rovers were less affected by environmental changes is borne out by some empirical data (Kaun et al., 2007).

Despite our agents' behavioural rules being fixed, and not responsive to environmental factors, they nonetheless modified their behaviours in response to environmental changes, such as the quality of resources in deeper layers of the substrate. This effect highlights how simple mechanisms of collective behaviour can allow social groups to efficiently adapt their behaviour to environmental parameters without requiring complex individual cognition (e.g., Couzin, 2009). Future extensions of our model could incorporate renewable food, similar to what is found in the wild, where fruit flies feed on live yeast (Stamps et al., 2012).

Our model agents are simplifications of fruit fly larvae, lacking many of the cognitive skills larvae are known to possess (Kahsai & Zars, 2011). For example, learning about specific locations in the environment, which our agents were not capable of, may influence clustering behaviours, possibly differently depending on behavioural phenotype (Kaun et al., 2007). Individual recognition of agents by others, which fruit fly larvae are capable of (Fisher et al., 2021; Khodaei et al., 2019; Khodaei & Long 2020), might allow clustering to be more efficient, if limited to kin. Furthermore, our model assumes that larvae have fixed metabolic and movement costs, though these may vary as larvae develop (Dombrovski et al., 2020; Merkey et al., 2011) possibly leading to increased clustering late in the third instar phase (Shoot et al., 2024). Our model therefore demonstrates that clustering behaviours, at least in principle, may not require these cognitive skills, although future studies might examine how adding some of them into the model alters the resulting clustering dynamics.

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Supplementary Materials

To \ From	Moving	Foraging	Clustering
Moving	If not alone, 0 If $S \leq 1$, 0.2 If $S \leq 11$, $1 - Ps$ If $S > 11$, 0.2	If not alone, 0 If $S \leq 1$, 0.2 If $S \leq 11$, $1 - Ps$ If $S > 11$, 0.2	If alone, 1 If $\Phi = 3$, 1 If $\Phi = 2$, 0.1 If $\Phi = 1$, 0.25
Foraging	If not alone, 0 If $S \leq 1$, 0.8 If $S \leq 11$, Ps If $S > 11$, 0.8	If not alone, 0 If $S \leq 1$, 0.8 If $S \leq 11$, Ps If $S > 11$, 0.8	0
Clustering	If not alone, 1 Else, 0	If not alone, 1 Else, 0	If alone, 0 If $\Phi = 3$, 0 If $\Phi = 2$, 0.9 If $\Phi = 1$, 0.75

Table S1: Agent state transition matrix for Model 1. The table gives the probability of transitioning from each state (columns) to each state (rows), as a function of environmental and individual variables. Φ = phase (or layer) of the current cluster. P_S = forage/move transition function (see main text).

Model parameter	Values taken	Cluster duration	Cluster size	Final food	Proportion time clustering
Visual range	2, **5**, 10	F(2, 15.19) **P < .001** η^2 = 0.002	F(2, 1649.05) **P < .001** η^2 = 0.03	F(2, 530.90) **P < .001** η^2 = 0.008	F(2, 1238.86) **P < .001** η^2 = 0.015
	Post-hocs:				
	2 *vs* 5	P = .12	P < .001	P < .001	P < .001
	2 *vs* 10	P = 1	P < .001	P = .11	P < .001
	5 *vs* 10	P = .44	P < .001	P = .044	P < .001
Metabolic cost	.025, **.05**, .1	F(2, 138.62) **P < . 001** η^2 = 0.05	F(2, 22.72) **P < . 001** η^2 = 6.36 x 10^{-4}	F(2, 531.83) **P < . 001** η^2 = 0.005	F(2, 348.97) **P < . 001** η^2 = 0.010
	Post-hocs:				
	.025 *vs* .05	P < .001	P = 1	P = 1	P < .001
	.025 *vs* .1	P < .001	P = .66	P =.025	P = .97
	.05 *vs* .1	P < .001	P = .34	P < .001	P < .001
Movement cost	.025, **.05**, .1	F(2, 43.92) **P < .001** η^2 = 0.004	F(2, 1.21) P = .30 η^2 = 2.78 x 10^{-5}	F(2, 589.69) **P < . 001** η^2 = 0.15	F(2, 368.67) **P < . 001** η^2 = 0.012
	Post-hocs:				
	.025 *vs* .05	P <.001		P < .001	P < .001
	.025 *vs* .1	P = .53		P < .001	P = 1
	.05 *vs* .1	P = .17		P < .001	P < .001
Speed	0.5, **1**, 2	F(2, 4208.38) **P < .001** η^2 = 0.05	F(2, 5291.55) **P < .001** η^2 = 0.13	F(2, 11992.33) **P < .001** η^2 = 0.073	F(2, 50481.29) **P < .001** η^2 = 0.59
	Post-hocs:				
	0.5 *vs* 1	P < .001	P < .001	P < .001	P < .001
	0.5 *vs* 2	P < .001	P < .001	P < .001	P < .001
	1 *vs* 2	P = .04	P < .001	P = .24	P < .001

Table S2. One-way ANOVA results for additional parameters varied in Model 1. The table gives ANOVA tables for each model parameter altered (rows) and each dependent variable measured (columns). The "Values taken" column lists all the values taken by the parameter, with baseline values bolded. Each cell gives the ANOVA F-test result, P-value for the F-test (with significant values bolded), and test power (η^2). Below are P-values for all post-hoc pairwise comparisons (where there is a significant main effect).

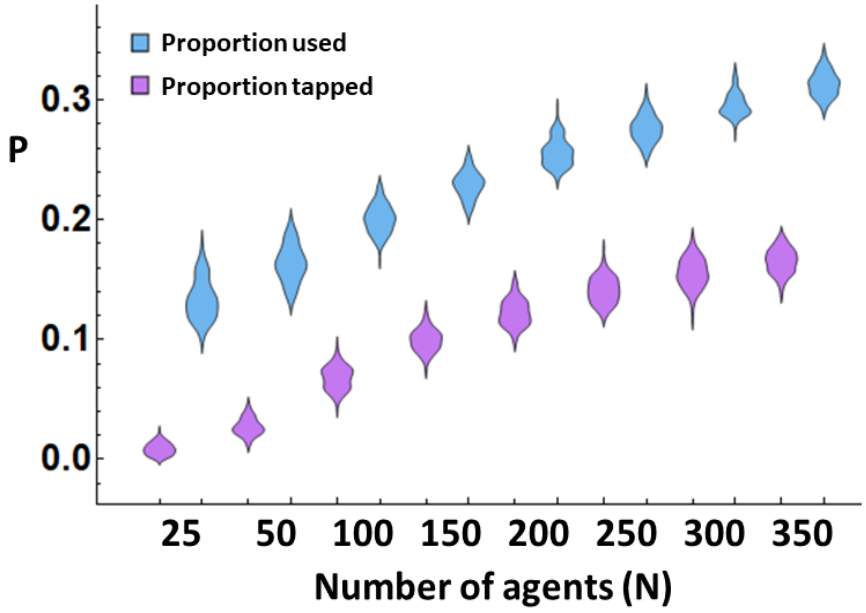

Figure S1. Violin plots of the proportion of all cells used for clustering (blue) and the proportion of those cells that reached phase 3 (that were depleted; purple) as a function of the number of agents in the simulation (N). The two data series have been slightly adjusted along the x-axis for clarity.

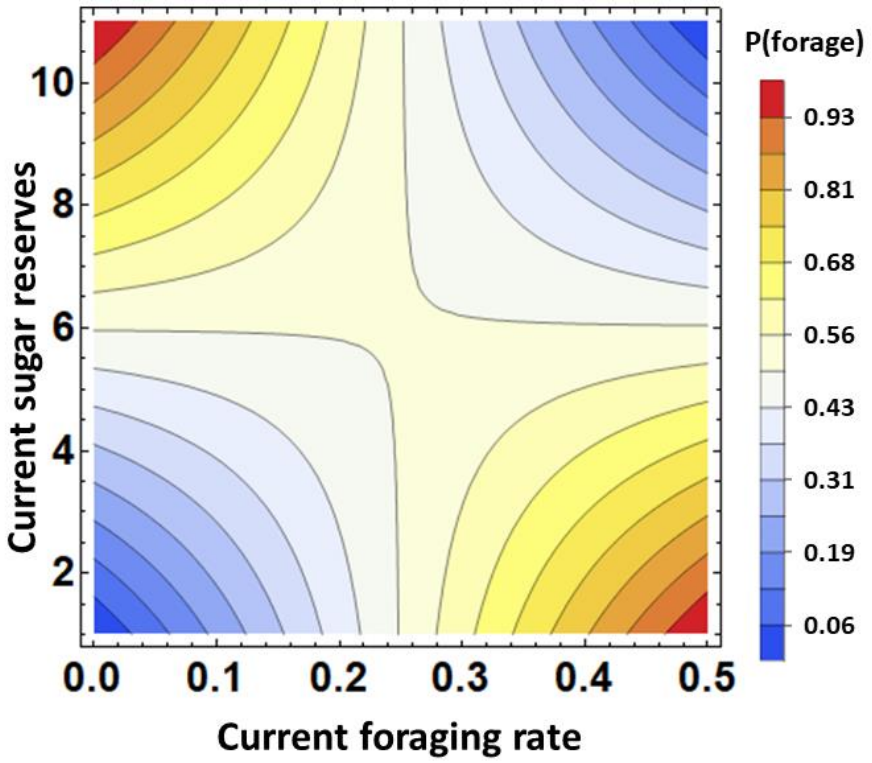

Figure S2. P_S, the decision-making function, as a function of the current surface foraging rate (which cannot exceed 0.5), and the agent's food reserves. Note that this function is only invoked if the agent's food reserves are between 1 and 11 (see Figure S1). P_S gives the probability of transitioning to or continuing to forage.

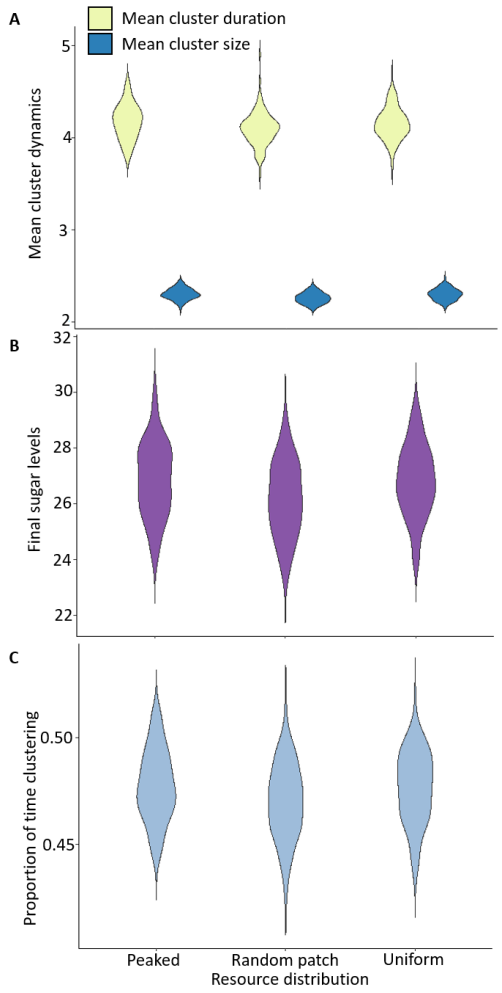

Figure S3. Consequences of altering the resource distribution. Violin plots of (A) mean cluster duration (yellow) and size (blue); (B) final food; and (C) proportion of time spent clustering, as a function of the spatial distribution of food on the surface (available without clustering).

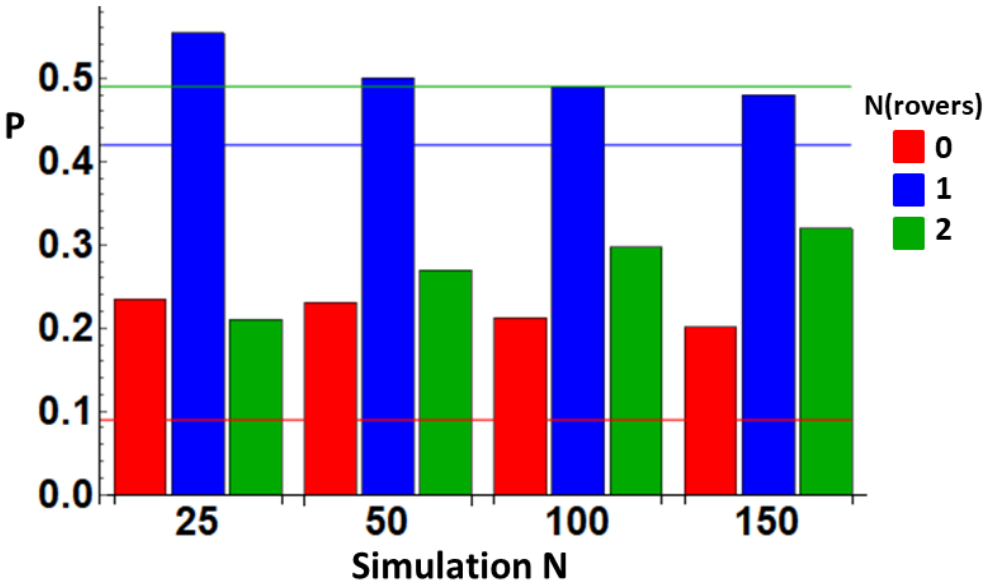

Figure S4. Under-representation of rovers in clusters in Model 2. The chart shows the proportion of all clusters of size N = 2 (the most common cluster size in Model 2) that had either no rovers (red), 1 rover and 1 sitter (blue), or 2 rovers (green), for simulations run with total agent numbers of 25 to 150 (x-axis). The horizontal lines represent the probability of finding a cluster with that number of rovers if agents were selected by chance (rovers make up 70% of the population). The chart shows that all-rover clusters (green) are far less common than expected, and clusters with one (blue) or no (red) rovers are more common than expected.

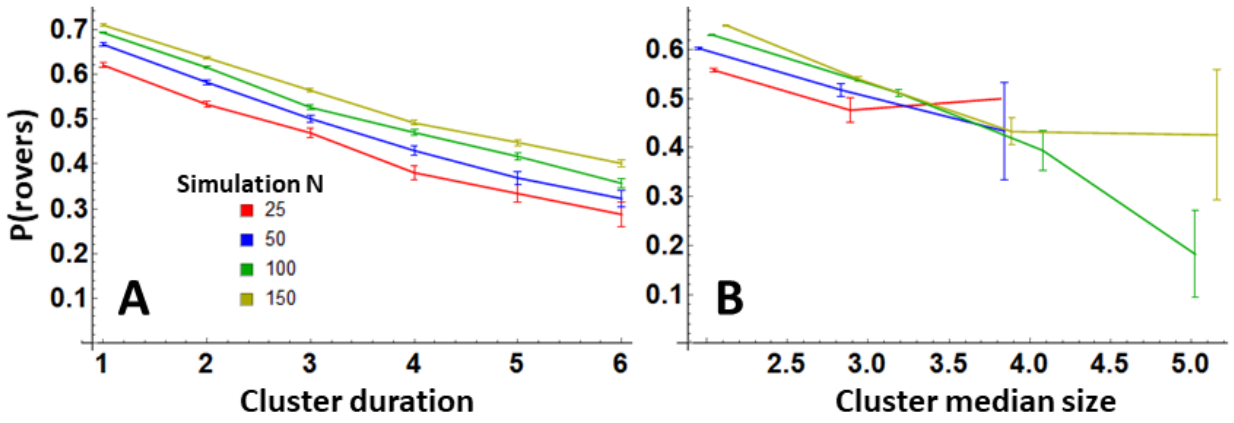

Figure S5. Cluster dynamics are affected by the proportion of rovers. **A**: proportion of rovers in a cluster (y-axis) as a function of the duration that the cluster persisted for (x-axis, in simulation timesteps) for simulations with N = 25 to 150 (line colour). **B**: proportion of rovers in a cluster (y-axis) as a function of the median size (in agents, across its lifetime) of the cluster (x-axis) for simulations with N = 25 to 150 (line colour). Error bars in both panels show ± SEM.

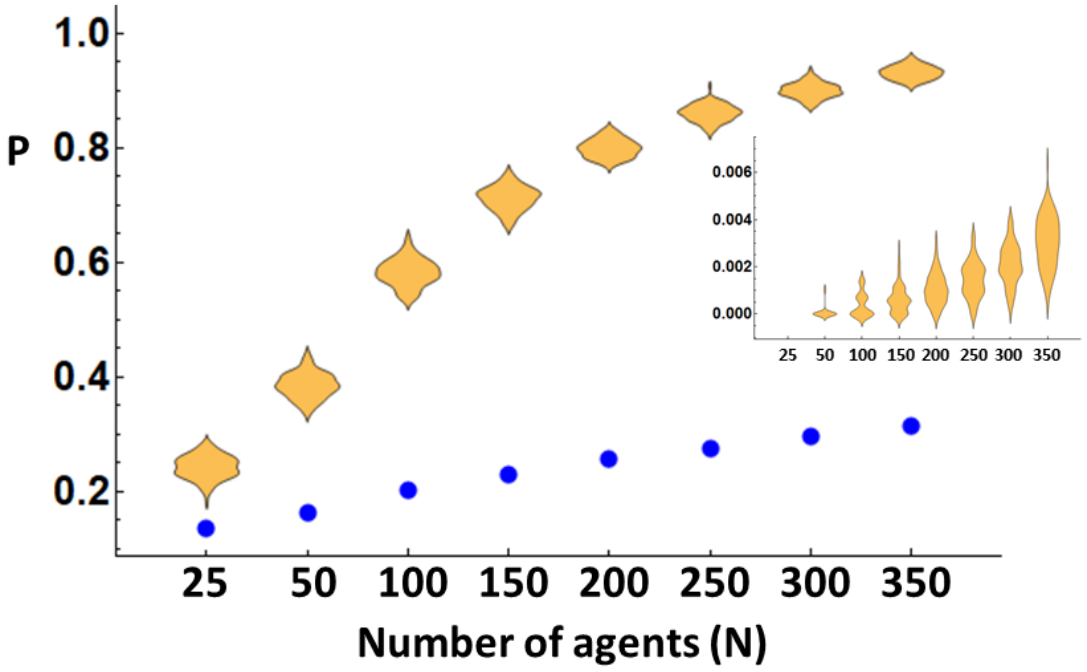

Figure S6. A violin plot of the proportion of all cells used for clustering (yellow) and the proportion of those cells that reached phase 3 (that were depleted; inset) as a function of the number of agents in the simulation (N) for Model 2. The corresponding mean data for Model 1 (taken from Figure S1) are shown as blue dots in the main panel. Axes for the inset are the same as for the main figure. The presence of rovers in the simulation spreads out clustering, so many more cells are clustered in and fewer are depleted (compare inset to Figure S1, purple data).

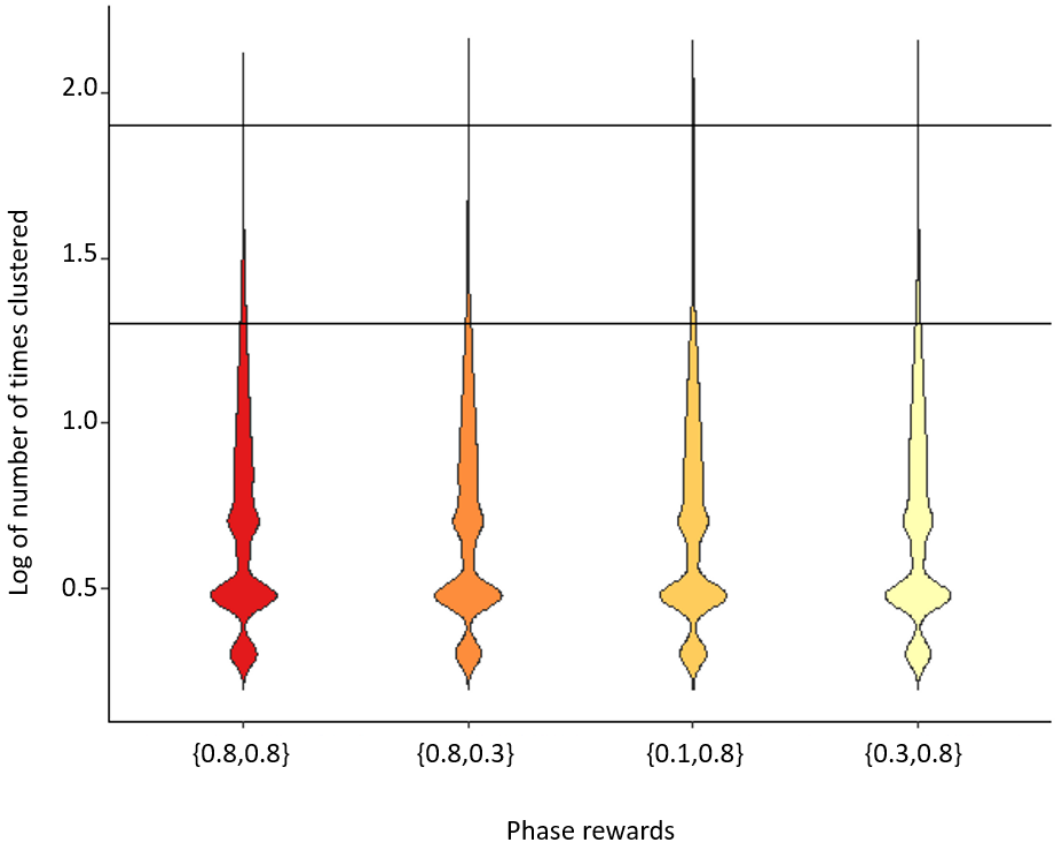

Figure S7. Violin plots showing the number of clusters that were in phase 1 (below the first black line), phase 2 (between the two black lines), and phase 3 (above the highest black line) in Model 2, as a function of the rewards obtained at each phase of clustering. Most clusters dissolve before reaching phase 2.