A genetic polymorphism affecting reliance on personal versus public information in a spatial learning task in Drosophila melanogaster

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Organisms that face behavioural challenges can use different types of information to guide their decisions. First, they can use the personal information they sample in their environment. Second, they can use the inadvertent social information provided by the behaviour of conspecifics or heterospecifics (i.e. public information). Currently, little is known about the interaction between genetic variation and the use of personal versus public information in natural populations. Here, we investigated whether a natural genetic polymorphism affects the use of personal versus public information in a spatial learning task in Drosophila melanogaster. We found that genetic variation at the foraging locus interacts with social context during spatial learning. While both allelic variants are able to use personal and public information to improve their navigation during 10 training trials, a probe trial revealed that individuals carrying the forR (rover) allele rely mainly on personal information, whereas individuals carrying the forS (sitter) allele either use or display more public information than rovers. Accordingly, transfer of social information is more important in groups of sitters than in groups of rovers. These results suggest that a positive feedback loop can occur between alleles promoting group living, such as forS, and the use and/or display of public information, ultimately providing the opportunity for the joint evolution of sociality and cultural traits.

1. Introduction

Learning, defined as a modification of behaviour with experience, appears as an adaptive and widespread process in animals. Learning allows individuals to face environmental heterogeneity in both space and time [1]. The genetic basis of variation in learning ability among closely related species and populations of the same species is an important avenue for research. Brains are complex machines that rely on the coordinated expression of vast genetic networks, both during development and throughout life [2–4]. Apart from genetic considerations, environmental factors are also critical to the process of learning. In particular, the social context in which a memory task is acquired or performed can significantly influence learning performance [5,6]. Social interaction with conspecifics or heterospecifics can provide useful information during the learning process. Instead of directly sampling their physical environment and acquiring personal information [7], individuals can gain information from others or provide social information to others, i.e. share social information [8]. Social information can be provided by conspecifics in the form of direct communication between individuals, but also as inadvertent social information, produced in the course of the animals’ own activities. When this inadvertent social information provides some cues about the quality of an environmental parameter, it is termed public information [7]. Public information may significantly reduce learning costs, such as adverse trial-and-error outcomes [9]. In some social species, including humans, an inability to benefit from social interactions can be severely deleterious (e.g. autism [10]). How genetic
factors and the social environment interact to influence learning, however, has been poorly investigated [10,11]. In particular, little is known about genetic variation in the relative use of public versus personal information. A plausible hypothesis is that genes favouring social interactions or group living may influence the proportion of social versus personal information used in the course of memory acquisition or retrieval. An increase in the number and diversity of social interactions could provide the basis for selection on the reliance on social information.

In this study, we use Drosophila melanogaster as a model system to investigate how genetic and social environmental factors interact during a spatial learning task. Our work is based on a well-characterized behavioural polymorphism that is due to a single biallelic gene in D. melanogaster. The foraging locus (for) encodes a cGMP-dependent protein kinase (PKG). It has two allelic variants—rover and sitter—that are distinguished by significant food-related behavioural differences in individual larvae and adults. Rovers bear at least one dominant forR allele and have higher PKG levels than sitters. Sitters are homozygous for the recessive forS allele [12]. Variation in PKG activity has been found to affect a wide range of behavioural and physiological responses [13,14]. Individual rovers leave food patches readily and explore more food patches, whereas sitters tend to aggregate and remain on food patches [15–17]. Consequently, sitters are expected to experience significantly more social interactions and, therefore, could be more sensitive to these interactions than rovers. In other species, the homologous for gene in ants and bees has been shown to regulate socially mediated foraging [18–20].

To test for a putative interaction between the foraging locus and social interaction in the context of learning, we used a spatial learning task recently developed for D. melanogaster [21,22]. In this task, individuals or groups of flies are trained to locate an invisible safe zone of optimal temperature within an aversively heated arena by using visual pattern cues on the walls or by observing the behaviour of other individuals. This task can be seen as the invertebrate version of the popular Morris water maze used in mammalian studies of cognitive defects and spatial memory [23,24]. Previous work showed that individuals [21] or groups of flies [22] can access the safe zone with increased efficiency over repeated trials and can use visual cues—such as geometrical patterns—to locate their position. To investigate the potential differential impact of social interaction between for genetic variants, we compared the reliance on public versus personal information in rovers and sitters by manipulating the information available to them.

2. Material and methods

(a) Fly strains

We used D. melanogaster rover (forR) and sitter (forS) natural allelic variants of the foraging (for) gene [16,25] kindly provided by M. B. Sokolowski. Flies were cultured on standard medium and always kept in groups at 22°C. To prevent escape from the arena during the experiments, 3- to 7-day-old adult female flies were anaesthetized on ice and their wings were clipped 12–24 h before they were introduced to the heat maze. Before the experiment, all individuals were maintained in small groups (less than 20 individuals) on standard yeast–cornmeal agar food under a 12 L:12 D cycle at room temperature.

(b) Spatial learning apparatus

We used a previously developed assay to decipher search strategies in D. melanogaster [21]. The ‘heat maze’ apparatus is an 18 cm diameter arena with a 5 cm high metal ring at its base that is covered by a thin layer of talc to prevent flies from escaping (figure 1a). This metal ring was surrounded by a 20 cm high, circular wall made of blank white paper that we used to display visual cues (four geometrical patterns, one per quadrant). The flies’ task was to reach a safe zone in an otherwise negatively reinforced environment, similar to the Morris water maze used in vertebrate studies [24,26]. The negative reinforcement in this case was elevated temperature. To keep the design simple and to control floor temperature in the area, we chose to use an array of 28 Peltier elements (arranged in a 5 × 5 grid; 24 measured 4 × 4 cm and four measured 2 × 2 cm; figure 1b). The surface temperature of each Peltier element was directly related to the amount of electrical power applied and was finely tuned via computer-control, assuring precision of less than 1°C. For 27 of the Peltier elements, the temperature was set to 37°C—a non-lethal negative reinforcement demonstrated in previous experiments that used this and other learning paradigms [27]. We used the remaining Peltier element (a small 2 × 2 cm Peltier) as the safe zone—equivalent to the platform in the Morris water maze—and set its surface temperature to a permissive 20°C. The 18 cm diameter arena thus consisted of a large ‘unsafe’ 37°C zone of approximately 250 cm² and a ‘safe’ 20°C platform of 4 cm² (figure 1). On top of this array of Peltier elements, we laid a thermally conductive plastic sheet as a floor surface. This surface allowed us to remove potential odour cues simply by washing the floor surface, to set up a very steep
temperature gradient between the safe and unsafe zones, and the probe phase. This experiment consisted of two phases: the training phase and the probe phase. Flies were trained over several trials—either alone or in groups of 10 flies—to become more efficient at locating the safe zone (see below). When alone, flies had access only to personal information—associating wall patterns to the position of the safe zone. When in groups, each fly could use the wall-pattern cues and/or inadvertent social cues provided by the other flies’ positions and movements.

To record the flies’ positions, we placed a commercial webcam and a circular light bulb on top of the apparatus (figure 1a). We video-tracked each fly’s position using ETHOVISION XT v. 8 (Noldus, Wageningen, The Netherlands). All data were deposited in the Dryad repository at http://dx.doi.org/10.5061/dryad.kt09v.

3. Experiment 1: learning alone versus learning in groups

In this experiment, we trained adult rover or sitter flies—either individually or in groups—to improve their efficiency at accessing the safe zone over successive training trials. Our first goal was to determine whether rover and sitter flies differ in how much they improve their performance in locating the safe zone over successive training trials, both when they are alone and when they are in a group. Our second goal was to investigate whether this improvement was essentially based on the personal information provided by wall-pattern cues (by providing a final trial where wall-pattern cues were rotated) or was facilitated by the social information provided by the presence of conspecifics (by comparing performance alone and in groups), and whether the two fly strains differed in what type of cues they preferably rely on.

(a) Experiment 1: methods

This experiment consisted of two phases: the training phase and the probe phase.

(i) Training phase

During the training phase, flies were subjected to 10 consecutive, 5 min trials during which the position of the wall patterns remained constant (figure 1c). Flies could therefore improve how quickly they detected the safe zone over trials by using the personal information provided by wall-pattern cues and/or the social information provided by the location and behaviour of conspecifics (in group treatments). Additionally, if rovers and sitters differ in their response to the social context in which learning takes place, then we would expect to find a significant three-way interaction between the ‘social environment’, ‘line’ and ‘trial’ factors. At the beginning of each trial, the single fly or the group of flies was simultaneously released in any quadrant except the one containing the safe zone (anywhere in quadrants 2–4; figure 1b). At the end of each trial, all flies were removed from the arena by gentle aspiration. Each trial immediately followed the preceding one.

(ii) Probe phase

The second phase, or the probe phase, immediately followed the last training trial and consisted of a single 5 min trial. Just before the probe phase, we rotated the wall patterns by 180° but kept the position of the safe zone constant (figure 1c). Thus, if flies associated the position of the safe zone with the wall-pattern cues during the training phase, we would expect their performance to decrease during the probe phase. By contrast, we would expect little or no effect on performance if flies were mainly using social cues—updating information about the new location of the target would be influenced by group size [29,30]. At the beginning of the probe phase, flies trained in groups were simultaneously released in any single quadrant except the one containing the safe zone (quadrant 1) or its opposite quadrant (quadrant 3).

In total, 36 individual rovers, 36 individual sitters, 24 groups of 10 rovers and 24 groups of 10 sitters performed the spatial learning task. In order to prevent any bias owing to the visual cue, we alternated star and diamonds patterns as the cue in the quadrant that contained the safe zone.

(b) Experiment 1: results

(i) Training phase

During the first trial, sitters tended to detect the safe zone faster than rovers, both in groups and alone (Cox regression, line: hazard ratio (95% CI) = 1.6 (1.2–2.27), p = 0.002; social environment: hazard ratio (95% CI) = 0.8 (0.52–1.30), p = 0.4; line × social environment: hazard ratio (95% CI) = 0.81 (0.42–1.56), p = 0.53). The lack of significant effect of ‘social environment’ and of the interaction ‘line × social environment’ would suggest that, when naive, rovers and sitters are not particularly influenced by the presence of other flies on the safe zone—they are not differentially using social cues to detect the safe zone. As the number of trials increased, flies became more efficient at accessing the safe zone (Cox regression, trial: hazard ratio (95% CI) = 1.06 (1.05–1.08), p = 10−3) and the time required to access this zone reached an asymptote after three to five trials (figure 2).

Importantly, this general pattern varied among lines and social environments (Cox regression, line × social environment × trial: hazard ratio (95% CI) = 0.84 (0.78–0.89), p = 0.03). In groups, both rovers and sitters improved their performance over trials (Cox regression, rover: hazard ratio...
(95% CI) = 1.07 (1.06–1.09), p < 10^{-3}; sitter: hazard ratio (95% CI) = 1.06 (1.05–1.08), p < 10^{-3}). Solitary rovers also became more efficient at finding the safe zone as the number of trials increased (Cox regression, rover: hazard ratio (95% CI) = 1.06 (1.02–1.1), p = 0.003), though the response was slightly slower than for flies trained in groups (Cox regression, social environment: hazard ratio (95% CI) = 0.73 (0.54–0.99), p = 0.046; social environment \times trial: hazard ratio (95% CI) = 0.97 (0.93–1.02), p = 0.30). However, despite a positive trend, solitary sitters never significantly improved their efficiency at finding the safe zone over time (Cox regression, sitter hazard ratio (95% CI) = 0.98 (0.94–1.02), p = 0.51) and they responded differently from those sitters trained in groups (Cox regression, social environment: hazard ratio (95% CI) = 0.79 (0.58–1.07), p = 0.13; social environment \times trial: hazard ratio (95% CI) = 0.90 (0.87–0.92), p < 10^{-3}). The hazard ratios for each trial were also never significantly different from the initial trial (Cox regression, comparison with trial 1, p > 0.06 for all trials 2–10).

(ii) Probe phase
Solitary rovers and sitters both exhibited a strong response to the rotation of wall patterns; they performed significantly worse during the probe trial than during the final training trial (figure 2; Cox regression, rover: hazard ratio (95% CI) = 0.37 (0.2–0.67), p = 0.001; sitter: hazard ratio (95% CI) = 0.35 (0.18–0.67) p = 0.002). Overall, these results suggest that, when alone, both natural genetic variants are able to use the information provided by visual cues to locate the safe zone, with an advantage for rovers; rovers progressively improved their ability to detect the safe zone over the first 10 trials. Rovers and sitters are thus capable of acquiring and retrieving physical information from the environment when this is the only type of information available.

Results differed significantly when learning took place in the presence of social cues. In groups, the response of rovers during the probe trial compared with the last training trial was similar to the response of rovers tested alone, i.e. groups of rovers
performed significantly worse during the probe phase than during their final training trial (Cox regression, group: hazard ratio (95% CI) = 0.70 (0.57–0.86), p = 0.001; comparison group versus alone: social environment: hazard ratio (95% CI) = 0.91 (0.65–1.2), p = 0.3; social environment × trial: hazard ratio (95% CI) = 0.71 (0.4–1.25), p = 0.24). Interestingly, sitters tested in a group detected the safe zone as efficiently as in the preceding training trial, even though the visual wall-pattern cues had been rotated (figure 2; Cox regression: hazard ratio (95% CI) = 0.94 (0.78–1.12), p = 0.72). Sitters tended to respond differently to the rotation of the visual patterns depending on whether they were in a group or alone (comparison group versus alone: Cox regression: social environment: hazard ratio (95% CI) = 1.02 (0.83–1.04), p = 0.09; social environment × trial: hazard ratio (95% CI) = 0.61 (0.33–1.1), p = 0.051). Despite a general trend, however, we could not detect a significant differential effect of the social environment (in group or alone) on how rovers and sitters respond to the change in visual cues (Cox regression: line × social environment × trial: hazard ratio (95% CI) = 0.56 (0.25–1.18), p = 0.12). These results would suggest that rovers mainly rely on wall-pattern visual cues to orient themselves, even in the presence of other flies. By contrast, sitters showed social facilitation during the learning process and, when in a group, social information was relatively more important for sitters than for rovers: the position of other flies mattered more to sitters than the position of the wall-pattern cues to locate the safe zone. Interestingly, when observing the first fly to access the safe zone (when no other flies were yet present), we found that the time required to access the safe zone increased between the final training phase and the probe phase in rover groups (ANCOVA with trial as covariate and group replicate as random factor; trial: F1,21 = 3.8, p = 0.03) but not in sitter groups (F1,21 = 1.003, p = 0.5). This suggests that sitter flies introduced in the maze may initially perform a distributed random search, independent of the wall patterns, that is then modified by social information provided by flies that progressively access the safe zone.

4. Experiment 2: use of pattern cues versus social cues during the probe trial

To measure the relative strength of the use of wall-pattern cues versus social cues, we performed an additional experiment using two heat maze apparatuses. In this experiment, focal flies were forced into conflict between using their personal information and using the social cues provided by the group during the probe trial.

(a) Experiment 2: methods

Two groups of flies (either two groups of rovers or two groups of sitters) were first trained simultaneously using the previously described training phase. One group was trained to locate the safe zone facing the star wall pattern; the other group was trained to locate the safe zone facing the diamond wall pattern (i.e. the opposite pattern in the arena; figure 3i). In each group, a single (focal) fly was marked on the thorax with a small dot of acrylic paint. The foraging activity of only this focal fly was video-recorded for each trial. At the end of the training phase, all flies were removed from the arena, all non-marked flies were released into their respective arena and the two marked flies were swapped from their initial arena into the other arena. Another trial was then performed without rotating the wall pattern. Thus, the position of the wall pattern no longer predicted the position of the safe zone for the focal fly, but it did for the other flies in the group. Fifty replicates were performed for each line.

From the results of experiment 1, the prediction for experiment 2 was that sitter focal flies would be less affected by the change in arena than would rover focal flies, because social cues appear more important for sitters than for rovers to locate the safe zone. It is important to note that, as in the first experiment, the genotypes of the focal fly and of the group flies were always the same (either rover or sitter). Hence, the factors ‘group genotype’ and ‘focal fly genotype’ are confounded, and it is not possible to distinguish the effect of use from display of social cues by different genotypes.

(b) Experiment 2: results

As previously observed, both rover and sitter focal flies within a group improved performance over the training phase (figure 3b; Cox regression: rover: hazard ratio (95% CI) = 1.08 (1.04–1.12), p < 10⁻³; sitter: hazard ratio (95% CI) = 1.06 (1.02–1.11), p = 0.001). The most important result of experiment 2 is that rovers and sitters were differently affected by the conflicting switch that occurred between the final two trials (Cox regression: line × trial: hazard ratio (95% CI) = 1.48 (1.10–1.99), p = 0.009). During the final trial in which focal flies were subjected to a ‘probe phase’, but the other flies of the group were not, rover performance significantly decreased but decrease in sitter performance was not significant—sitters located the safe zone as quickly as they had in the last training trial (figure 3b; Cox regression: rover: hazard ratio (95% CI) = 0.61 (0.38–0.97), p = 0.03; sitter: hazard ratio (95% CI) = 0.9 (0.5–1.33), p = 0.43). During the first minute of this last trial (the probe trial), rovers also tended to spend significantly more time than sitters on the quadrant that had previously predicted the location of the safe zone (mean ± s.e.: rover: 13.7 ± 1.5, sitter: 8.2 ± 1.7; paired sample t-test: t = 4.74, p < 10⁻²). These results confirm that, during the training phase, rovers primarily improved performance by using wall-pattern cues, whereas sitters primarily improved performance by progressively using or displaying social cues. These results also provide information about the strength of these associations. Within a rover group—despite the fact that most flies performed well during the previous trial—either the focal fly still did not use its social group as an information source or the rover group did not provide social information. Similarly, within a sitter group—despite the fact that the focal fly faced environmental change—its performance remained the same. We conclude that sitters either progressively used public information or that sitter groups provided more public information than did rover groups.

5. Discussion

The present experiments suggest a genetic influence on variation in the reliance on personal versus public information. Our results show that rovers improved their ability to detect a safe zone over trials by associating their position with wall-pattern visual cues; they were only slightly sensitive to the social context. By contrast, sitters did not show much improvement in their ability to find the safe zone when they were
trained alone, but showed strong social facilitation when in a group. Two equally plausible and not exclusive hypotheses may explain why sitter flies improve more in group contexts. First, it is possible that sitter flies are better at using inadvertent social information—i.e. by paying progressively more attention to the position and behaviour of other flies in the arena—than are rover flies. According to this hypothesis, sitter flies rely more on public information than do rover flies. Second, it is possible that sitter flies’ behaviour changes in some marked way upon entering the safe zone, and that the focal fly (irrespective of its genotype) learns this change in behaviour, but that the behaviour of rover flies does not change so markedly. According to this hypothesis, sitter flies display more public information than do rover flies. To distinguish between these hypotheses of differential use or display of public information between sitter and rover flies, it would be necessary to test the spatial performance of focal flies within groups of flies of the alternate genotype.

Whether this genotypic difference in behaviour affects the use or display of public information, these experiments demonstrate that sitters trained in a group outperform even the best lone learners, as expected from previous work on the benefits of collective behaviour over individual action [29,30]. Variation in the foraging gene activity thus appears to affect the type of information used or displayed by individual flies. Differential reliance on personal or public information

Figure 3. Experiment 2: use of pattern cues versus social cues during the probe trial. (a) Schematic of the experimental protocol. (b) Spatial learning performance of rover and sitter focal individuals trained in groups, measured in time to locate the target. Rover and sitter performances are represented with grey circles and black triangles, respectively.
may depend on their relative costs. When alone, sitters performed the learning task poorly and thus may benefit greatly from the social cues available when in a group. On the other hand, rovers performed the task well when alone, by using personal information. The presence of conspecifics provided little improvement. One should note that the observed difference between rovers and sitters is not due to a simple innate difference in aggregation tendency. When naive, rovers and sitters performed equally well. It was only over training trials that sitters progressively increased their reliance on available public information. How individuals estimate their performance, the value of the different sources of information and the nature of the underlying neural mechanisms that allow this behavioural choice must still be elucidated.

These results are consistent with the hypothesis that allelic variants that promote group living and recurrent social interactions, such as the for allele in *Drosophila* [15–17], can be associated with increased use and/or display of social information. Genes that favour the reliance on social information and those that favour group living are expected to co-evolve, because they positively impact each other. Previous studies show that the benefits of using social information increase with the number of potential informers [30–33], which is influenced by genes that promote social interaction. Reciprocally, the benefits from social interaction are expected to be higher when use of social information improves [8,34,35]. This kind of positive feedback loop between genes that promote social interaction and genes that govern the use of social information can help us understand the seemingly concomitant evolution of sociality and culture in animals [8,36,37]. In particular, quantitative models predict that phenotypic evolution can be greatly enhanced when there is a positive interaction between reciprocal traits [38–41].

Our learning experiment also illustrates that reliance on social information may be an advantageous strategy in changing environments. Indeed, as floor temperature was kept constant during our probe trial (including the cooler safe zone), this trial can simulate a rapid environmental change, with the correct target located in the opposite quadrant during the probe trial relative to training. In this case, we observed that the greater relative importance of public information for sitters enabled them to rapidly switch their response towards the new goal, whereas rovers paid the cost of spending more time searching within a zone that had become noxious. The timing of environmental change (i.e. environment predictability) is particularly important when evaluating the relative costs and benefits of relying on social versus personal information [42]. Collective behaviour arises from the combined tendency to form groups and to use social information that is available in those groups; this use of social information is expected to result in accurate decision-making in situations with a lot of uncertainty [29,43]. In the context of spatial heterogeneity, it is probably that immigrants should learn to copy the behaviour of local individual; paradoxically, rover individuals, which are more likely to be immigrants, do not pay more attention to social cues than do sitter individuals, as shown in our experiments.

Most studies highlight a trade-off in the use of social versus personal information [35,37,44]. In the case of *D. melanogaster*, the for locus probably does not directly mediate such a putative trade-off. First, our experiment suggests that the genetic polymorphism at the for locus is not strictly associated with reliance on either social or personal information. Sitters are also able to use personal information (resembling the ‘critical social learner’ phenotype; [45]), and rovers can benefit from the presence of conspecifics during training (resembling the ‘conditional social learner’ phenotype; [46]). Second, the for genetic polymorphism has already been shown to be highly pleiotropic (e.g. to incur negative frequency-dependent selection at the larval stage when food resources are low; [25]). Therefore, allelic frequencies at the for locus are probably under various selective pressures and, as such, do not directly reflect a putative trade-off between personal versus public information use in *Drosophila*. As noted earlier, a promising line of research would be to investigate putative differences in quality/quantity of information being provided by groups; for instance, by studying the behaviour of focal individual flies in alternate variant groups of flies.

The pleiotropic nature of the for locus [13,25,47] illustrates the intricate nature of the evolution of social, cognitive and physiological traits. The fact that the for locus, which was originally isolated for its regulation of foraging behaviour [12,14], also impacts the use or display of socially acquired information does not come as a surprise. In bees, sociality may have evolved through modification of gene networks that regulate several aspects of behaviour and physiology, such as foraging (including the orthologous for locus; [18–20]). Finally, the for locus has been shown to influence a variety of other learning processes, such as associative olfactory learning [48], visual pattern memory [49] and retroactive interference [50]. Our results suggest a strong interaction between genetic and social environmental factors and appeal for a re-evaluation of previous conclusions on the effect of the for polymorphism on learning, as learning assays are typically conducted either alone (e.g. visual pattern memory; [49]) or in groups (e.g. associative olfactory memory; [48]).

This work is an early attempt to link a naturally occurring genetic polymorphism, an ecologically relevant learning task and the social context in which the learning task is performed. Our study illustrates that allelic variants can interact with social context to promote a positive feedback loop between group-forming behaviour and social information use, and that they provide the basic ingredients for joint natural selection of sociality and cultural traits.

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References


