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Social competition stimulates cognitive performance in a sex-specific manner

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Primer design

Primers were designed with a melting temperature of 60℃ +/- 1℃ and a CG content between 20-80%. Primer pairs were tested for efficiency using a 10 times dilution series of whole-body RNA and accepted if the efficiency fell between 90 and 110% with an $R^2 > 0.98$ (Table S1).

Visual learning and memory analysis

Visual learning tests were performed on individual flies with their wings clipped to prevent escape. The assay tested the ability of flies to associate a visual cue with a “safe” temperature zone [1, 2]. An array of 25 Peltier elements (4x4 cm elements in a 5x5 grid) were arranged so as to create an arena floor covering 20x20 cm. On top of this was placed a metal ring 18 cm in diameter and 6 cm high and covered in talcum powder to prevent fly

escape. Directly outside the metal ring was a piece of white cardboard to remove external visual cues. Each Peltier element temperature was precisely controlled by the application of a current. 24 elements were heated to 37℃ +/- 1°C while one “safe” element was heated to 20℃ (Figure S8A). Elements heated to 37℃ acted as a non-lethal negative reinforcement for the flies. On top of the array, a sheet of white paper was laid to remove all mechanosensory cues not relating to temperature and was replaced every trial to remove olfactory cues. A light source directly above the arena standardised light conditions. Flies positions were recorded with a commercial web camera (Logiteck C920 HD Pro 1080p) and flies were video tracked using MatLab (Figure S8B).

Flies were tested for their ability to associate a “safe” Peltier element with a visual cue, a green dot on the floor of the arena that was placed in the centre of the “safe” area. A red dot was placed diametrically opposite on a Peltier element heated to 37℃. Flies were then introduced into the arena for 3 trails of 5 min. Between each trial, flies were rested on a paint brush above the arena floor but within the confines of the blank paper for 10 sec. At the start of each trial, flies were placed haphazardly on the arena floor and then their movement followed for 5 min. Learning was assessed by the time a fly took to spend 20 sec consecutively in the “safe” zone, an amount of time taken to reflect a decision having been made by the fly [2]. Learning index was calculated as:

LI = Time taken to find “safe” zone trial 1 – Time taken to find “safe” zone trial 3

Learning was controlled by the total distance moved by each fly (cm) outside of the safe zone throughout each trial. Flies that had learnt to associate the visual cue with the “safe” zone should orientate more quickly towards it in subsequent trials and therefore not increase the total distance moved during a trial. If flies increased their total distance moved it would show that they a “safe” zone was present but could not correctly orientate towards it. The difference between the distance moved during the first test and distance moved during the last test was assessed by taking the difference between the first and last trials and

comparing these to 0 (or no difference between trials). A one sample t-test was used to do this.

To fully test learning, a further 5 min trial was conducted but with the visual cues switched, so that the green dot was now on a heated Peltier element and the red dot was lying in the “safe” zone. When assessing this “probe” trial, time taken to spend 2 sec in the nominal “safe” zone was used. This was due to the speed at which flies moved from the green dot when realising that this no longer represented the “safe” zone. Mean distance from “safe” zone throughout the trial was also used to assess learning. The learning index for each fly was calculated as:

Probe index = Time taken to find “safe” spot memory trial / Average time for control males to find “safe” spot

Probe index = Distance from “safe” zone memory trial / Average distance from “safe” zone of control males

Videos were coded such that the observer was blind to the social treatment identity of the male. Conspecific and heterospecific exposure were tested concurrently by matching each fly in a social exposure treatment to a fly held singly as a control. These focal flies were assayed one after the other, to control for time of day effects, and so the probe “learning” index of a fly was always controlled by a matched single fly.

Supplementary figures

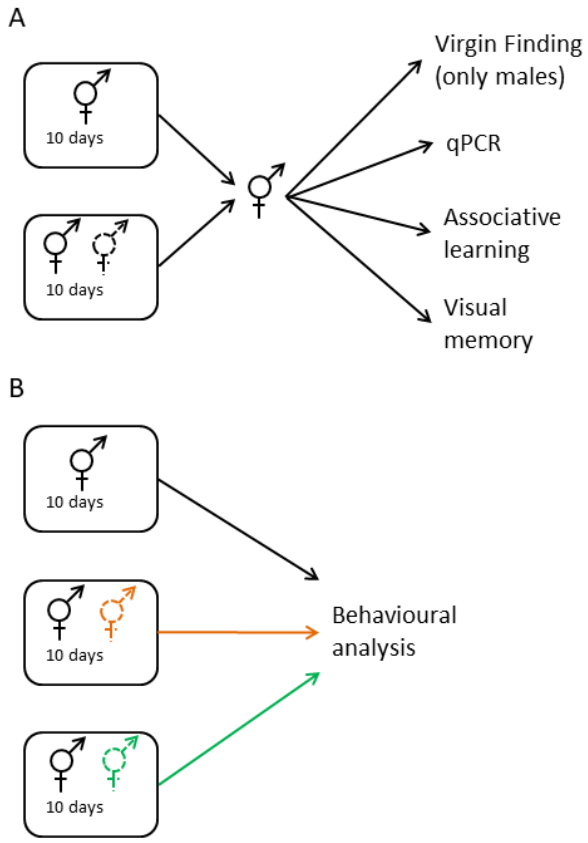

Figure S1: Experimental design. Social treatments were composed of no competitor, same-sex conspecific competitor, or same-sex heterospecific competitor. Focal flies were always D. melanogaster. a) focal males and females were placed singly or with a social partner (either conspecific or heterospecific – dotted sign) before undergoing one of either virgin finding, gene expression analysis, associative learning, or visual memory b) behavioural analysis was undertaken on focal individuals (black) kept singly, with a conspecific social partner (orange) or with a heterospecific social partner (green). Analysis was undertaken while social partners were still present.

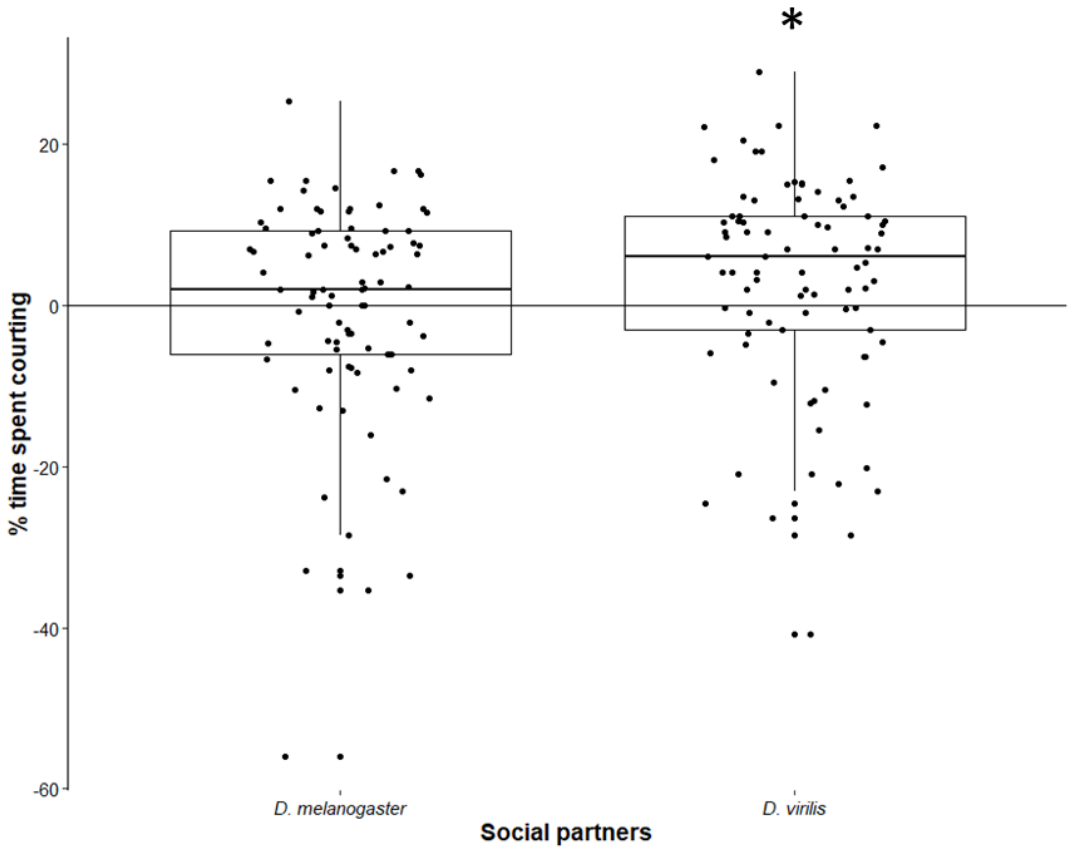

Figure S2: Virgin-finding assay - time spent courting. The percentage of time males spent courting females any female having been when kept with either a conspecific or heterospecific partner. This was standardised by time-matched single males, hence compared to 0 * $p < 0.05$. D. melanogaster males kept with conspecific rivals did not significantly change courting effort (AOD: $X21 < 0.0001$, $N = 165$, $p = 0.986$).

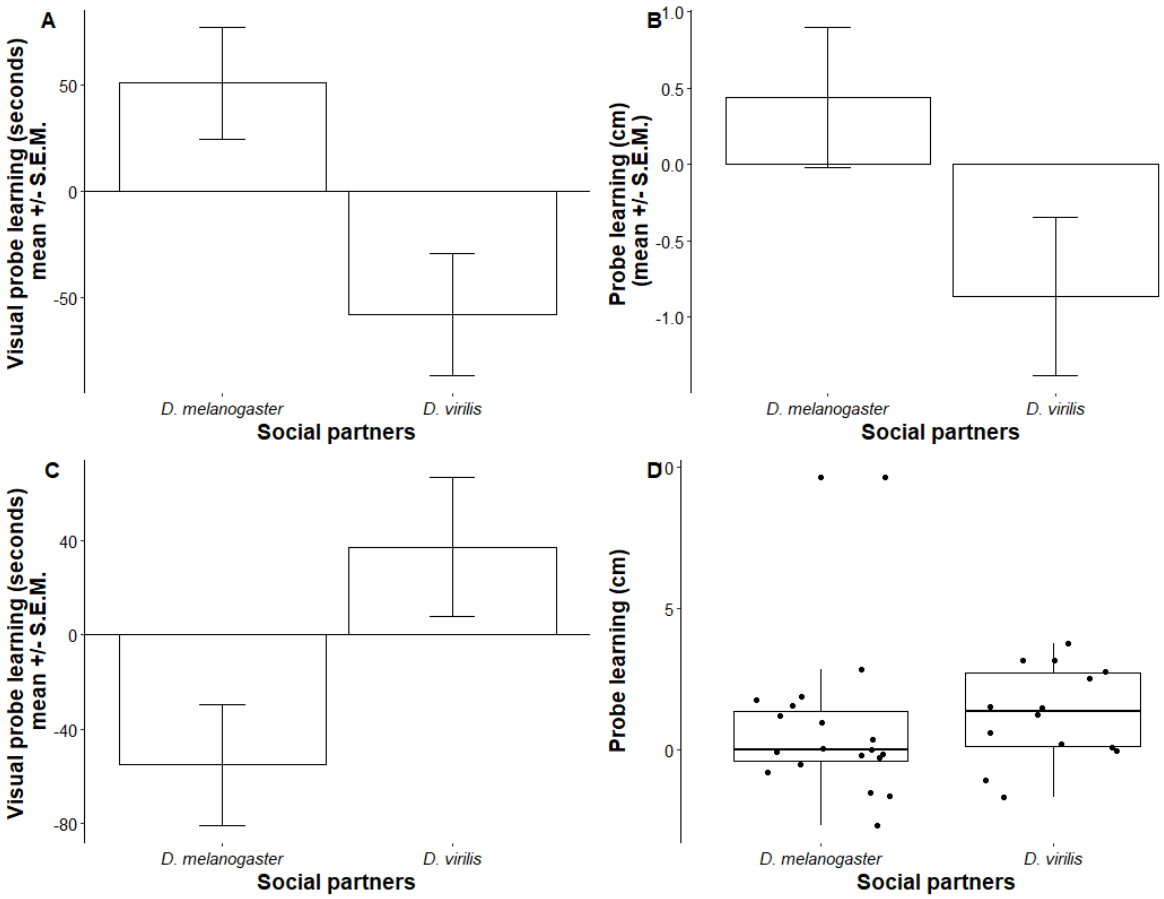

Figure S3: Visual probe reversal learning ability shown as the time spent in the “safe” zone for males (A) and females (C) and the average distance from the “safe” zone in the probe trial for males (B) and females (D). All individual flies were controlled for time of day and day effect via comparison with a single fly.

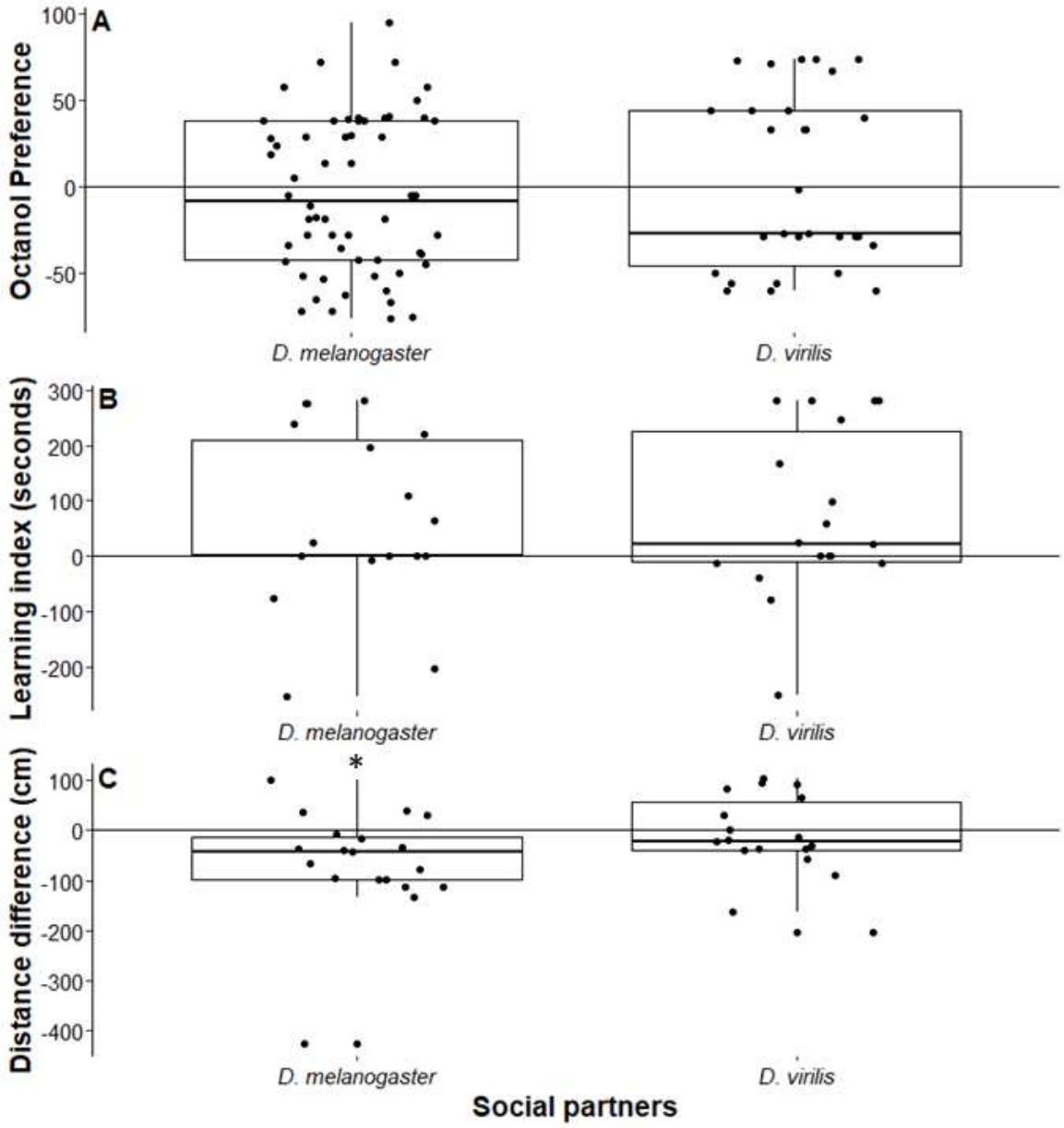

Figure S4: Male innate olfactory preference (A), visual learning (B), and distance travelled in visual assay between trial 1 and 3 (C). Points represent individual data points. There was no change in male ability to sense Octanol compared to single males in the olfactory learning assay regardless of social stimulus (conspecifics: $z = 0.844$, $N = 58$, $p = 0.399$ heterospecifcs: $z = 0.453$, $N = 30$, $p = 0.650$). There was no difference in visual learning depending on a male's social partner (MW: $z = 0.382$, $N = 40$, $p = 0.718$). There was a significant decrease in the distance travelled outside the "safe" zone between trial 1 and 3 for males kept with conspecifics ($X^2 = -2.575$, $N = 19$, $p = 0.01$) but not for males kept with heterospecifics ($X^2 = -0.457$, $N = 18$, $p = 0.647$). Importantly, males did not increase the

distance travelled with increasing trials as this would suggest males learnt searching behaviour instead of to direct movement towards a visual cue.

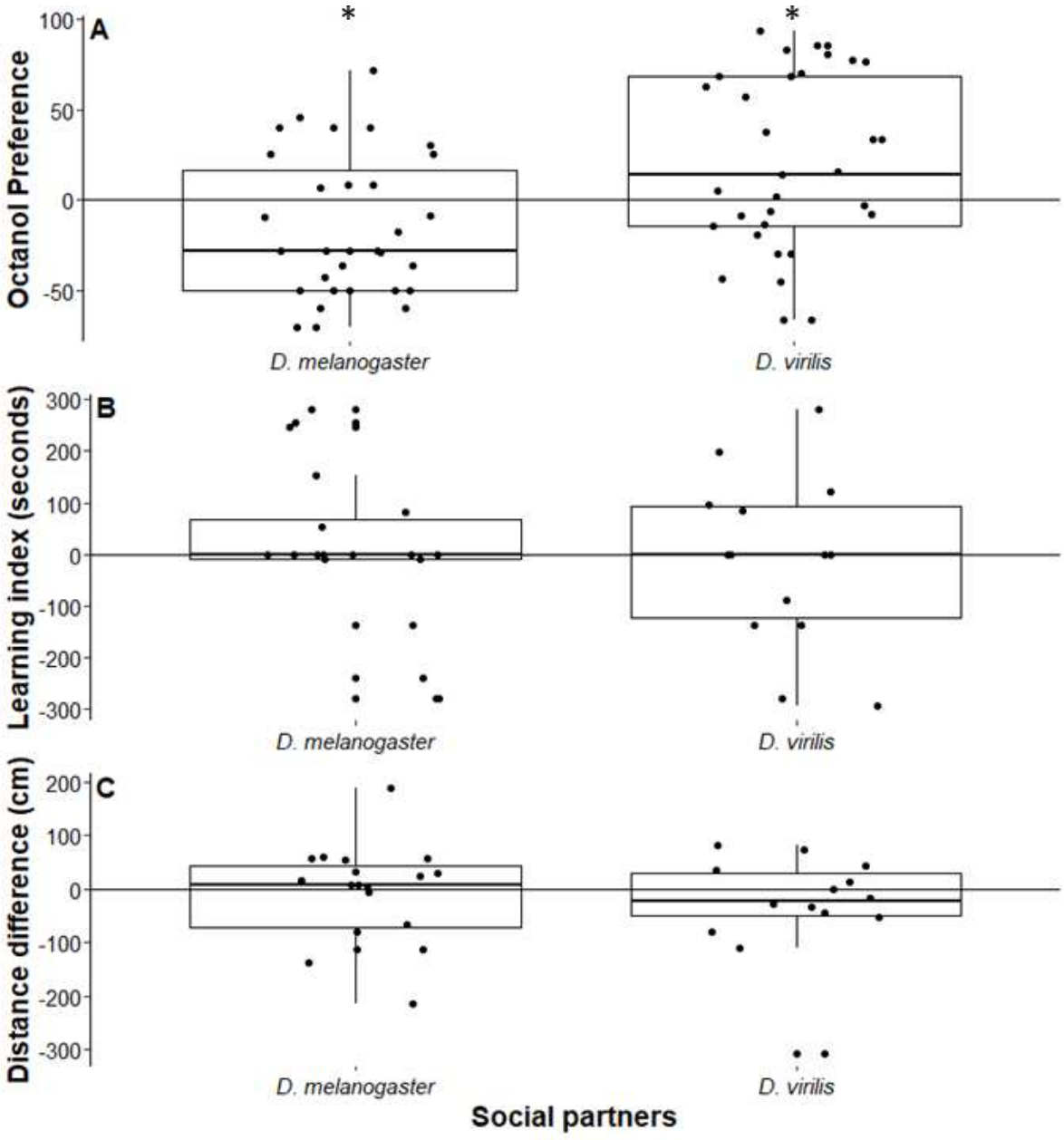

Figure S5: Female innate olfactory preference (A), visual learning (B), and distance travelled in visual assay between trial 1 and 3 (C). Females differed in their olfactory preferences when kept with both social partners compared to females kept singly (conspecific: $z = 2.079$, $N = 31$, $p = 0.038$ heterospecific: $z = 2.010$, $N = 33$, $p = 0.044$). There was no difference in visual learning depending on female housing with social partners ($z = 0.068$, $N = 35$, $p =$

0.961). The distance females travelled outside of the safe area did not differ between trial 1 and 3 regardless of the social stimulus (conspecific: X^2 = -0.121, N = 19, p = 0.0904 heterospecific X^2 = -0.973, N = 14, p = 0.0331). Importantly, females did not increase the distance travelled with increasing trials as this would suggest males learnt searching behaviour instead of to direct movement towards a visual cue.

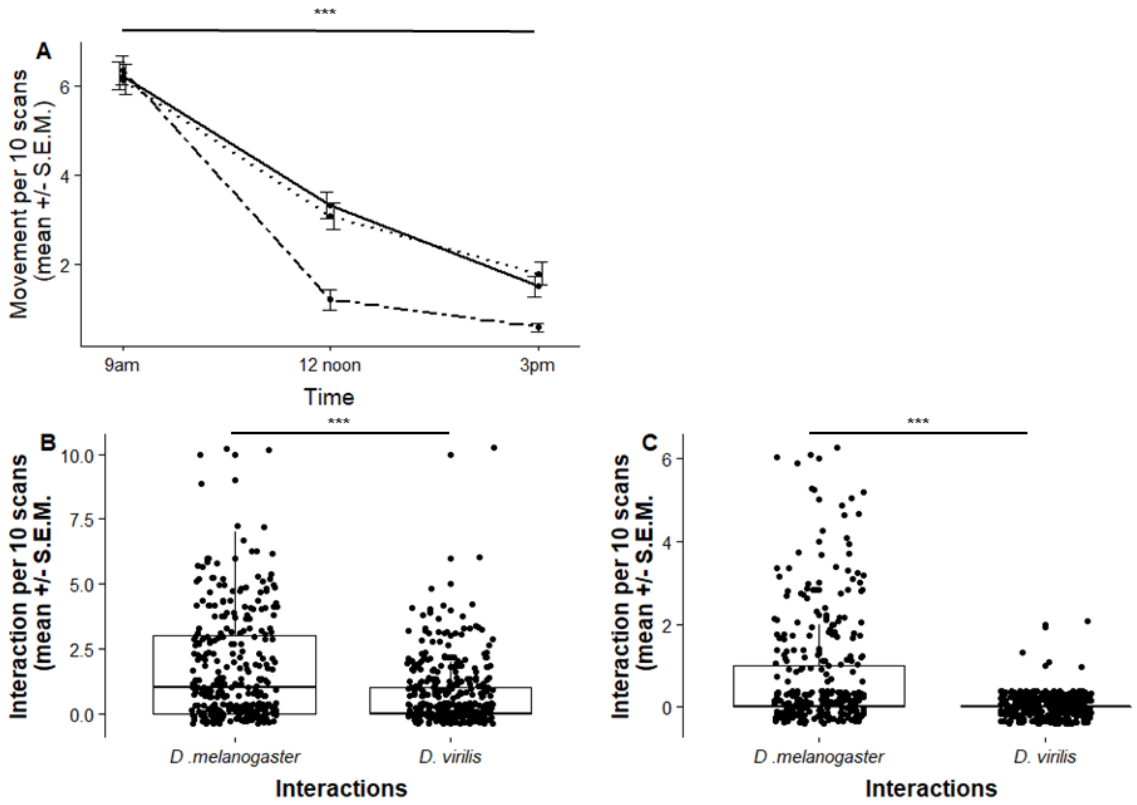

Figure S6: Behaviour in social treatments – males. Focal flies were held singly, with a conspecific rival or a heterospecific partner for 10 days, and behavioural scans were made each minute for 30min on day 6, 8, and 10 at 9am, 12pm and 3pm. A) Proportion of time (mean +/- S.E.M) spent moving in behavioural scans for flies held singly (solid line), with a heterospecific (dotted line) or a conspecific social partner (dashed line). For paired flies the B) proportion of scans in which flies were within one body length or had C) aggressive interactions with conspecific or heterospecific social partners. *** p < 0.001

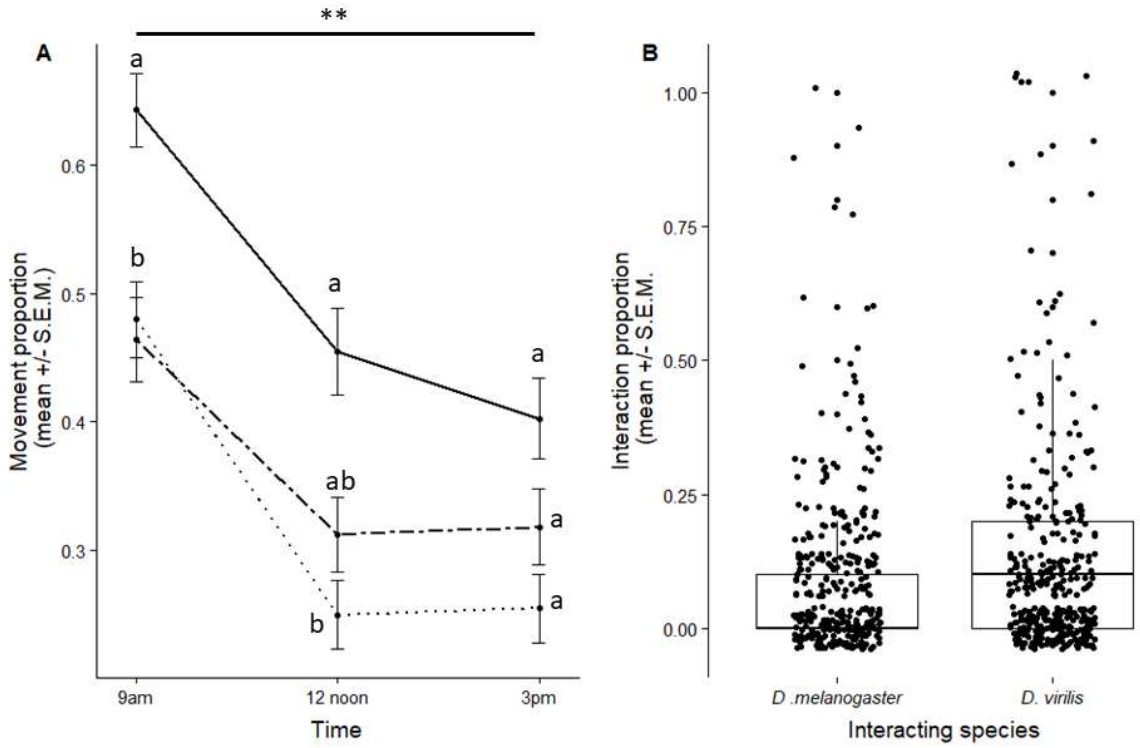

Figure S7: Behaviour in social treatments – females. Focal flies were held singly, with a conspecific rival or a heterospecific partner for 10 days, and behavioural scans were made each minute for 30min on day 6, 8, and 10 at 9am, 12pm and 3pm. A) Proportion of time (mean +/- S.E.M) spent moving in behavioural scans for flies held singly (solid line), with a heterospecific (dotted line) or a conspecific social partner (dashed line). Within time periods significance is defined by letters. For paired flies the B) proportion of scans in which flies were within one body length. ** $p < 0.01$

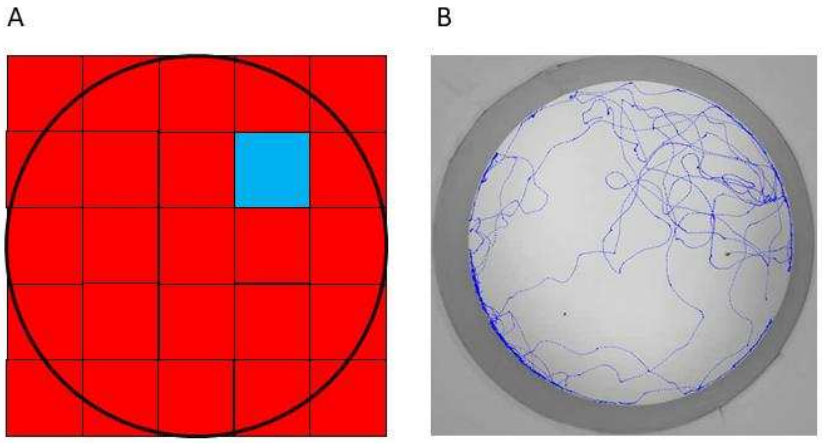

Figure S8: Visual learning and memory equipment. a) Representation of hot and cold areas in the Peltier array. Each Peltier element was 4x4 cm^2. Red represent elements heated to 37^oC, the blue element represents an element kept at 20^oC (the designated “safe” zone). b) Example of tracking performed by Matlab with dots representing the “safe” areas and “non-safe” areas in the same orientation as a fly’s movement over 5 minutes represented by the blue line

Table S1: qPCR Primer sequence showing forward and reverse nucleotide sequence.

Gene	Forward	Reverse
E1f	GTCTGGAGGCAATGTGCTTT	AATATGATGTCGCCCTGGTT
Rap21	TTCACTTACGAACCATCAAACATT	GCTGGCTGACTTCCTTTCAC
Brp	GACATCAAGGACCGCAAGAT	GCCATATCCACCTGGTTGTC
Futsch	ACGTTTCCGATTGTCACGTC	GCTGCTACCTCCTCATCGTC
Neurexin	GACAACAACTGGCACACGAT	TACTGTGGCGACCCAGAAT

Supplementary references

1. Ofstad T.A., Zuker C.S., Reiser M.B. 2011 Visual place learning in Drosophila melanogaster. Nature **474**(7350), 204-U240. (doi:10.1038/nature10131).

2. Foucaud J., Burns J.G., Mery F. 2010 Use of Spatial Information and Search Strategies in a Water Maze Analog in Drosophila melanogaster. Plos One **5**(12). (doi:10.1371/journal.pone.0015231).