Immediate social context and early social experience differentially modulate female sexual receptivity in Drosophila melanogaster

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Abstract

Most organisms rely on social interactions for reproduction and survival. These interactions can influence how individuals perform in a certain immediate social environment but they may also impact subsequent behaviour in the long-term. As reproductive related behaviours have substantial influence over fitness outcomes, female sexual receptivity, the likelihood to accept mating, should be adjusted to the social environment. Here we investigated the effect of immediate social context and early long-term social experience on *D. melanogaster* female virgin and post-mating receptivity. Virgin female receptivity is measured as latency to first mating and post-mating receptivity is measured as latency to first remating and number of copulations in 24h. We find that when the immediate social context is increased (mating in a group versus mating in a single pair) female post-mating receptivity increases, but virgin receptivity is unaffected. This effect is mediated via volatile compounds located on the surface of flies, most likely cuticular hydrocarbons, many of which have established pheromonal properties in this species. Long-term exposure to females during the early stages of adulthood decreases both virgin and post-mating receptivity later in life, compared to females raised in isolation. This long-term social experience is influenced by three female sensory modalities; vision, touch and olfaction. Immediate social context and early social experience, therefore, have opposite effects, affect different expressions of receptivity and are determined through different sensory mechanisms.

Keywords

Female receptivity, social context, pheromones, cuticular hydrocarbons, social isolation, social experience, sensory modalities.
**Introduction**

Social interactions are fundamental to the lives of most species, from microbial aggregates to human society (Frank, 2007). Living in groups provides several benefits to the individual both directly such as the acquisition of mates and communal rearing of offspring (Duménil et al., 2016; Lin et al., 2015; Lof et al., 2009; Wertheim et al., 2002a; Yang et al., 2008) and indirectly such as the diffusion of predation risk and an increase in food gathering. Living in group does, however, also bring challenges as group size is positively correlated with competition for resources, prevalence of rivals and increased risk of infection (Côté and Poulin, 1995; Meunier, 2015; Patterson and Ruckstuhl, 2013). Thus, functioning in a group is an intricate balance between cooperation and conflict for each individual. Individuals determine whether to cooperate or engage in conflict through innate processes and individual experiences. For example, most organisms instinctively know how to interact; display sexual behaviours in one situation and aggressive behaviours in another (Billeter and Levine, 2013; Fernández et al., 2010; Fernández and Kravitz, 2013). However, when situations are more ambiguous these behaviours are modulated by experiences. Males, for instance, become less sexually active after recurring rejection (Bastock and Manning, 1955; Shohat-Ophir et al., 2012), preventing them from courting a hopeless target. When social experiences are lacking, however, this can lead to negative consequences as for example in humans who show increased aggression after being socially isolated (Cacioppo et al., 2010; Hawkley and Capitanio, 2015; Nino et al., 2016). When these modulations of behaviour occur early in life they are often long-lasting and irreversible, like social isolation during adolescence in rats which leads to long-lasting changes in anxiety and depressive behaviours and altered neuronal circuits in adulthood (Burke et al., 2017). This suggests that there are critical periods early in life in which innate programs and experiences converge to adjust behaviour to the social context. However, what mediates these effects remains poorly understood.

The social life of the fruit fly *D. melanogaster* offers a paradigm in which to study the effect of immediate social context and early long-term social experience. Fruit flies aggregate on food sources (Becher et al., 2012; Lin et al., 2015; Zhu et al., 2003), where they engage in social interactions such as mating (Spieth, 1974) and aggression (Fernández and Kravitz, 2013) and allows females to lay eggs communally (Duménil et al., 2016; Lin et al., 2015; Lof et al., 2009; Wertheim et al., 2002a; Yang et al., 2008), which increases larval survival (Etienne et al., 2002; Lof et al., 2009; Wertheim et al., 2002b). We can therefore utilize this model organism, with its extensive toolkit of genetic manipulations and behavioural assays (Laturney and Billeter, 2014; Linder and Rice, 2005; Sirot et al., 2015; Yamamoto et al., 2014), to start unravelling the mechanisms instructing social experience and its effect on social behaviours.

Indeed, fruit flies respond to social context. For example, when males experience immediate exposure to rivals, they increase aggression (Fernández et al., 2010), decrease mating duration (Bretman et al., 2009) and increase sperm allocation (Garbaczewska et al., 2013). When females experience increased immediate social context, increased number of group members or group diversity during mating, they mate more often (Billeter et al., 2012;
Ellis and Kessler, 1975; Gorter et al., 2016; Krupp et al., 2008). After early long-term exposure to same-sex individuals, however, both sexes decrease aggressive behaviours (Hoffman, 1990; Liu et al., 2011; Svetec and Ferveur, 2005; Ueda and Kidokoro, 2002) and take longer to engage in mating (Bretman et al., 2009; Ellis and Kessler, 1975; Kim and Ehrman, 1998). This suggests that, in *D. melanogaster*, immediate social context and early long-term experience can have opposite effects on aggressive and reproductive behaviours.

One important trait for reproduction is female sexual receptivity, the likelihood to accept mating. Female receptivity should be under tight regulation as there are both benefits and costs associated to the number of matings (Arnqvist and Nilsson, 2000; Bateman, 1948). Benefits of mating include higher lifetime reproductive success (Arnqvist and Nilsson, 2000; Gowaty et al., 2010; Jennions and Petrie, 2000; Lefevre and Jonsson, 1962; Priest et al., 2008), while costs observed are reduced lifespan and immunity (Chapman et al., 1995; Kamimura, 2007; Kuijper and Morrow, 2009; Kuijper et al., 2006; Rice et al., 2006; Schwenke and Lazzaro, 2017). The environment influences these costs and benefits due to availability of resources and mates. Female receptivity is modulated accordingly by immediate food availability and social context (Billeter et al., 2012; Gorter et al., 2016; Krupp et al., 2008). Here, we focus on the impact of immediate social context and early social experiences to understand how this influences female receptivity and whether social experiences can modulate these traits.

As *D. melanogaster* relies on chemosensation to communicate, especially during reproduction, we hypothesise that chemical cues from conspecific are a readout of social context. Flies are attracted by pheromonal cues to places with or visited by other flies (Bartelt et al., 1985; Duménil et al., 2016; Keesey et al., 2016; Lebreton et al., 2012; 2017a; Lin et al., 2015; Wertheim et al., 2002a). This attraction is mediated by the male aggregation pheromone, 11-cis-vaccenyl acetate (cVA) (Bartelt et al., 1985; Keesey et al., 2016; Lin et al., 2015; Wertheim et al., 2002a) which impacts male-male interactions by acting as a proxy for male density (Liu et al., 2011; Wang et al., 2008). Other compounds that function as pheromones in fruit flies are cuticular hydrocarbons (CHCs) (Ferveur, 1997). These are compounds produced by specific cells on the cuticle of flies (Billeter et al., 2009) that can be transferred to other flies both via air and physical contact (Farine et al., 2012; Lebreton et al., 2017a). The CHC profile of both sexes includes many different compounds and have also been shown to play a role in attraction (Dweck et al., 2015b; Everaerts et al., 2010; Lebreton et al., 2017a). More specific effects are attributed to 7-tricosene (7-T), a CHC produced by both sexes but in higher quantities by males. 7-T is stimulatory for female receptivity (Grillet et al., 2006). Aside from chemosensation, other sensory modalities might be involved in determining social context as well, both touch and sight are, for example, implicated in the regulation of social behaviours (Bastock and Manning, 1955; Markow, 1987). Additionally, acoustic and olfactory cues are suggested to interact to stimulate females (Rybak et al., 2002). Therefore, several sensory modalities are hypothesised to influence female receptivity during immediate social context and early social experience.
Here, we have manipulated the social experience of females at two stages of their adult life to determine the impact on female sexual receptivity. First, the immediate social context is investigated by testing single male-female pairs or groups of six males and six females. Second, early long-term social experience is investigated by isolating or grouping females directly after eclosion. We find that immediate and long-term social experience have opposite effects on female receptivity. Experiencing group conditions at the time of mating leads to an increase in female post-mating receptivity. Whereas, after early long-term experience with a same-sex group, females decrease both virgin and post-mating receptivity, compared to females raised in social isolation. Immediate social context is experienced via volatile pheromones excreted by the other members of the group. The pathway to determine early social experience most likely includes vision, touch and olfaction.

**Material and methods**

**Drosophila stocks**

The wild-type strains were Canton-S (CS), Oregon-R (OR) and w1118. For smell, hearing, vision and touch impairments, we obtained the following stocks from Bloomington Stock Center or as stated otherwise: Orco+ (w Orco+:+, #23129, (Larsson et al., 2004)), Inactive (iav;+;+) gifted by Joel Levine (O'Dell and Burnet, 1988), NinaE17 (w;sr1,ninaE17,e;+, #5701, (OTousa et al., 1985)) and Piezo ko (w;piezo ko;+, #58770, (Kim et al., 2012a)).

**Rearing conditions**

Flies were reared in a 12:12 hr light/dark cycle (LD 12:12), lights on at 09:00 local time (Zeitgeber time 0), at 25 °C. The rearing food contained: agar (10g/L), glucose (33 g/L), sucrose (15 g/L), yeast (35g/L), cornmeal (15g/L), wheat germ (10g/L), soya flour (10 g/L), molasses (30 g/L), propionic acid (5 ml of 1M) and Tegosept (2g in 10ml ethanol). This medium is referred to as “Fly food” in this report. Virgin adults were collected using CO₂ anaesthesia and aged in same-sex groups of 20 in food vials for 5-8 days. Flies were raised in the same light and temperature conditions as rearing.

**Manipulations of early long-term social experience**

Female flies were raised in different conditions for 7 days on fly food. Females were raised in same-sex groups of 20 in fly food vials of height (h) 95* diameter (d) 25 mm (volume = \( \pi(0.5d)^2h = 4.7*10^4 \ mm^3 \)). Females raised in social isolation were separated in small glass vials of h 40 * d 8 mm (volume = 2.0*10³ mm³) with fly food or in fly food vials of h 95 * d 25 mm. Any additional group sizes and raising manipulations were carried out in fly food vials of h 95 * d 25 mm. Different concentrations of fly pheromones, extracted as described below, dissolved in hexane to a total volume of 400 µL was added on top of the fly food on the day of collection. Hexane was evaporated from the vial by use of nitrogen gas within 2 min. Vial partitioning was accomplished by adding a clear or solid blue pvc sheet (Kangaro,
300 mmicro) fitted snugly in the middle of the vial, illustrated in supplementary figure 1A and 1B. The partition was either sealed off directly by a nitrocellulose plug (scientific laboratory supplies, 25mm) or a mesh partition and plugged, illustrated in supplementary figure 1C.

Antennal surgery
The third segment of the antennae or the arista of female flies were surgically removed. On the day of eclosion female flies were separated and moved to anaesthesia by ice. With two pairs of fine forceps the whole third segment of the antennae or the arista from at its basis specifically were removed at both sides of the fly head. Females were then moved to vials with food and raised either in a group or in isolation as described above.

Fly pheromone extraction
Fly pheromones were extracted from mixed-sex Canton-S groups. Flies were collected at day of eclosion and kept on food for 5-8 days after which they were frozen at -18°C in empty rearing bottles. The number of flies was determined by weight with an approximate weight of 0.001 gram per fly. On the day of hydrocarbon application, the frozen flies were extracted with hexane (Chromasolv, Sigma) in bulk with 10 μL hexane per fly and shaken for mild agitation for 3 minutes. After this, the solution of hexane and pheromones was divided over the experimental vials (described in method section “Manipulations of early long-term social experience”) or filter papers (described in method section “Fly odour experiments”).

Behaviour assays
All assays were conducted in the presence of fly food and in the same light and temperature conditions as raising and rearing. Red light was utilized to monitor behaviour during the dark phase. Single pairing assays were conducted in small Petri dishes of 35x10 mm with 3 ml of food poured on the bottom as described in (Gorter and Billeter, 2017), for group mating assays, 6 virgin females followed by 6 virgin males were placed into a larger Petri dish of 55 x 13 mm. In short, the test subject(s), virgin female of indicated genotype, and corresponding male(s), wild-type Canton-S, were aspirated into the Petri dish. All experiments began in the active phase in the afternoon between Zeitgeber time (ZT) 7 and 9. Webcam cameras (Logitech B910 webcam using the SecurityMonitor Pro software [Deskshare]) took pictures of the dishes at 2-min intervals for 24 hour to score the virgin mating latency, number of matings and latency to 1st remating (Billeter et al., 2012; Krupp et al., 2008). For a subset of the experiments up to two hours at the beginning were video recorded for a more accurate measure of virgin mating latency. The number of matings was scored as start time of each mating. The virgin mating latency was calculated as time of first mating minus start time of the experiment (time male was inserted and dish placed under the camera). The remating latency was defined as start time of second mating minus start time of first mating. If no second mating occurred remating latency was defined as end time of experiment minus start time of first mating. For the group assays the total mating frequency was counted and divided
by 6, the virgin latency was averaged for the first 6 matings that occurred and the remating latency was averaged for the latency between mating 1 and 7, 2 and 8, up to 6 and 12. Flies that did not mate at all were excluded from all abovementioned analyses. Any outliers were excluded based on [based on average±(standard deviations*3)]. This was less than 10% of the replicates.

Fly odour experiments
To subject flies in the mating assays to fly odours we used an airpump system as described previously (Gorter and Billeter, 2017; Gorter et al., 2016). In short, pressurized air was guided through a bottle containing a source of odour or an appropriate control substrate for the whole duration of the experiment. Subsequently the air was split towards 40 mating arenas after each odour source. Two sources of odour were tested. First, odour of alive flies was tested in 500 ml glass bottles layered with 100 ml fly food. The day before the experiment between 1500 and 2000 mixed-sex Canton-S flies of 5-8 days old were collected in one glass food bottle and attached to the airpump system. On the day of the experiment the odour flies were transferred to a clean glass food bottle and reattached to the airpump system. Second, pheromone extracts of flies were tested. On the day of the experiment, extract of 7000 flies with hexane was added to a 50-ml glass bottle. Hexane from both the experimental and control bottle was evaporated with nitrogen gas within 45 minutes directly before the start of the experiment. Tubing used for these experiments was Tygon with 4.8 mm inner diameter, which decreases the loss of molecules due to the smoothness of the material. All arenas were tested in an air-regulated cabinet (Gorter and Billeter, 2017).

Additionally, we performed an experiment with different dosages of fly pheromone extract on filter paper within the mating arena. Pheromone extract with solvent was pipetted in five concentrations (extract of 0, 4, 10, 16 and 22 flies with 10 µL hexane per fly) on square filter paper (Munktell Ahlstrom, 65 g/m²) of 25x25 mm. The hexane was evaporated in plain air and the filter paper was added to the mating arena within 45 minutes after pipetting and directly before the start of the experiment.

Statistical analysis
Differences in female receptivity, virgin latency, latency to first mating and number of copulations, were statistically analysed with the lme4 package in R (Team, 2015). Social condition (mating or raising) was included as fixed effect and date was included as random effect whenever applicable, to account for day-to-day variation, in mixed effects models. When both the fixed and random factor were included, the best fitting mixed effect model was selected by backwards elimination of the non-significant fixed effect using log-likelihood ratio tests. When only a fixed effect could be included the results of a general linear model between two groups at the time was recorded. For survival, both time and raising condition were included in a model as fixed effects and their interaction was tested, the best-fitting model was selected. Normal distribution of residuals was inspected visually and data was tested on homogeneity of variances with Levene’s test. Whenever the data did not pass either
one of the assumption, a log transformation was performed, if necessary followed by a Z transformation. Mate choice was tested with a binomial test in R for significant incidence of either one of the raising conditions. The results are presented in supplementary table 1. The courtship index of males towards females of both raising conditions was tested with a Wilcoxon signed rank test in graphpad prism 5 (GraphPad Software, Inc.).

**Results**

**Immediate social context sensed through pheromones increases post-mating receptivity**

Immediate social context can be comprised of exposure to different group sizes, genetic diversity and presence of males and females. Previous research has shown that females increase the number of copulation in 24 hours in response to increased group size and group diversity (Billeter et al., 2012; Gorter et al., 2016; Krupp et al., 2008). Here, we wanted to further document the effect of group size on female receptivity. We investigated wild-type Canton-S (CS) females with CS males in immediate social contexts of equal sex ratio and measured the effect on virgin mating latency, latency to first remating and number of copulations in 24h. We found that it takes longer for the virginal mating to take place within a group context (6 males and 6 females CS) compared to a single pair context (1 male and 1 female CS) as virgin mating latency was significantly slower in groups than single pair (figure 1A). After this initial mating, however, post-mating receptivity is increased in groups compared to single pairs, measured as latency to first mating (figure 1B) and number of copulations in 24h (figure 1C). Flies achieved the first mating faster in single pairs, but had more remating when tested in groups. Due to previous results we now attribute these effects to females as it was shown that female genotype accounts for 47 percent of variance in number of copulations in 24h, while the male genotype only accounted for 11 percent (Billeter et al., 2012), nonetheless, at this stage, we cannot exclude any male effects on the timing and number of copulations.

Females require a functional classical odorant receptor repertoire in order to respond to genetic diversity in their social context and increase their receptivity (Billeter et al., 2012). We tested the hypothesis that social context in terms of group size is detected via volatile chemical cues. We subjected single male-female CS flies to an airflow passed through a bottle with wild-type male and female CS on a food source. Single male-female pairs tested in an airflow coming from a group of flies did not change virgin mating latency (figure 1D) or latency to first remating (figure 1E). However, the number of copulations was increased when pairs were exposed to odours from other flies (figure 1F). We therefore conclude that volatile fly odours are one cue through which social context enhances post-mating receptivity.

For further determination of what fly air compounds enhance post-mating receptivity, we extracted pheromones from mixed groups of CS flies and passed these via an airflow through mating arenas with single male-female CS pairs. As for fly air, virgin mating latency was unaffected by pheromone air (figure 1G). Post-mating receptivity was enhanced with a borderline significant decrease in latency to first remating (figure 1H) and a clear
increase in number of copulations (figure 1I). Additionally, when male-female CS pairs were mated in the presence of a filter paper with pheromone extract from different group sizes, these extracts from various group sizes increased virgin latency (figure 1J) but only the extract from a group of 10 flies increased post-mating receptivity measured as number of copulations (figure 1K and 1L). Taken together, these data show that volatile fly pheromones signal the presence of a group of flies, leading to an increase in post-mating receptivity.

Figure 1: Social context, sensed through pheromones, increases mating Dot plots with average and error bars SEM of virgin mating latency or an average of the first 6 mating latencies in minutes (A), latency to first remating or an average of the first 6 rematings in hours (B) and number of copulations or the total number divided by 6 in 24h (C) for 1 male and 1 female or 6 males and 6 females CS, housed together for 24h. Virgin mating latency in minutes (D, G), latency to first remating in hours (E, H) and number of copulations in 24h (F, I) for 1 male and 1 female, housed together for 24h in the presence of odours from life flies (D-F) or fly pheromone extract (G-I). P-value of mixed effects models reported when no condition effect was present. Post-hoc analyses reported with significance stars. N=23-26. Virgin mating latency in minutes (J), latency to first remating in hours (K) and number of copulations in 24h (L) for 1 male and 1 female, housed together for 24h in the presence fly extract in concentrations mimicking group conditions. Significance stars, or p-value when bordering significance, reported for conditions differing from control (1x1) tested with general linear models. For detailed statistics see supplementary table 1. N=27-30. * p<0.05, ** p<0.001, ***p<0.0001.
Early long-term social experience decreases female receptivity

Flies in the previous experiments were communally raised in same-sex groups prior to the receptivity measurements and thus had the same early social experience before being immediately transferred to mating chambers with different social contexts. To understand the effect of early social experience, females were either isolated or grouped from the day of eclosion to the day of testing. To test how general the effects of early social experience are, we tested females from three commonly used strains, namely CS, Oregon-R (ORR) and w1118, with a communally raised CS male. Females from all three strains increased virgin mating latency as a result of social experience compared to social isolation (figure 2A). Interestingly, only CS and w1118 showed decreased post-mating sexual receptivity after early social experience, both in time to first remating (figure 2B) and number of copulations (figure 2C). ORR lacks this response and has the lowest post-mating receptivity with a time to remating of about 18 hours (figure 2B) and on average 2 copulations in 24 hours (figure 2C). This suggests that the post-mating receptivity of ORR females is more robust and less sensitive to early long-term social experience than that of CS and w1118. Overall these data show that female social isolation increases sexual receptivity, with a faster initiating of mating as well as higher continuation of mating.

Communal raising occurs in same-sex groups of 20 individuals in tubes with a total volume of 4.7*10^4 mm^3, which gives the individuals an approximate volume of 2.3*10^3 mm^3 per fly. To match the group situation as closely as possible in all other aspects the social isolation condition is performed in small tubes with a volume of 2.0*10^3 mm^3. To check whether the effect is due to the number of flies present, we used different densities during early adult life for CS females. Social density ranged from one single fly to 2, 5, 10 and 20, all raised in 4.7*10^4 mm^3 tubes. Females raised in isolation decreased virgin latency (figure 2D) and latency to first remating (figure 2E) as well as increased number of copulations (figure 2F), but not significantly as a consequence of the sample size in combination with the small effect sizes. The effect of group experiences seems to occur at different densities for latency to virgin mating versus post-mating measurements. Virgin mating latency is short for small groups (5 and 2 individuals) as well as singly raised (figure 2D). While both latency to first remating and number of copulations seem directly affected by the addition of a second individual and the effect is the most extreme in the largest group of 20 (figure 2E and 2F). This suggests that the effect of early social experience is due to number of individuals and not the availability of space or food. Consecutive matings are increased due to social isolation, while virgin receptivity seems more density dependent.
To further understand how early social experience influences this female mating behaviour, we investigated whether it is due to changes in female receptivity or female attractiveness eliciting an increased courtship response from males. To determine whether females become more attractive after social isolation, CS males were presented with a headless female, to exclude the influence of female behaviour, from each raising condition at the same time. With this assay the males did not show a preference to either female as both female types were courted equally (figure 2G) even though singly raised females get a higher percentage of first matings when a male is in the presence of an intact female from both conditions (figure 2H). These results confirm that the increase in sexual behaviour is due to female receptivity to
males and not a change in attractiveness. Additionally, we tested whether females accept any male faster after social isolation, irrespective of the male’s quality. For this experiment we chose males mutant for both the yellow and white gene ($y^w, w^r$) since they are poor courters, initiate courtship later, and most importantly, need longer active bouts of courtship than wild-type males to achieve mating with wild-type females (Bastock, 1956). If females are more willing to accept these less stimulating males, this suggests a lack of choosiness and implies reckless or maladaptive mating behaviour. We found an increase in virgin mating latency for socially isolated females with a control CS male, but not significant (figure 2I). The latency to virgin mating is increased with $y^w, w^r$ males for both females with and without early social experience, showing the impact of male courtship on virgin receptivity (figure 2I). However, this latency is shorter for socially isolated females (figure 2I), this suggests that isolated females accept any male faster. Together, these experiments show that females become more sexually receptive after isolation and imply that these females become less choosy and engage sexual activity in situation where group raised females do not.

**Different sensory modalities play a possible role in determining early social experience**

Females are more sexually receptive after social isolation than after early long-term social experience. We next explored which sensory modality (hearing, olfaction, vision and touch) females need to determine their social raising context. Hearing ($iu^1$), olfaction ($orco^1$) and touch (piezo$^{KO}$) mutants all showed a decrease in virgin mating latency after social isolation when tested with a communally raised CS male, whereas for blind ($ninaE^{17}$) mutant females social experience did not affect virgin mating latency (figure 3A). This suggests that a functioning visual system is important for the influence of early social experience on virgin receptivity. For post-mating receptivity, we were unable to show any decrease of time to first remating or increase in number of copulations after social isolation (figure 3B and 3C). This can either be explained by the genetic background of these flies or it could mean that all senses are involved in mediating post-mating receptivity in respect to long-term social experience. Interestingly, piezo$^{ko}$ mutants show an almost significant decrease in post-mating receptivity after social isolation (figure 3B and 3C), suggesting that touch might be necessary to increase post-mating receptivity in response to social isolation. To further investigate the effect of vision and touch, we reared flies in partitioned vials. For vision, we separated single flies from a group of flies by a clear or a dark partition to determine whether seeing other flies is sufficient for the group effect (illustrated in supplementary figure 1). For touch, we reared single flies in a vial with a clear partition in the middle and a mesh partition on top (illustrated in supplementary figure 1). On the other side of the vial was either a group of flies or no flies to see the effect of being raised in complete isolation or while hearing, smelling and seeing (but not touching or tasting) a group. The effect on virgin receptivity in this experiment lacks statistical support due to small sample size (figure 3D) and post-mating receptivity shows no effect at all, possibly due to the use of a smaller group size during raising than previously (group of 6 versus group of 20, figure 3E and 3F). However, the observations on virgin mating latency support the influence of vision as well as touch for determining early social
exposure (figure 3D). For vision, females isolated from a group by a clear division show a lesser decrease in latency than flies separated by a dark division, suggesting that seeing other flies might diminish the effect of social isolation. For touch, females able to see, smell and hear others show the same response as completely isolated individuals compared to females reared in the group at the other side (figure 3D), which suggests a necessity for touch or taste to determine social experience.
Figure 3: Involvement of sensory modalities in determining social experience Dot plots with average and error bars SEM of virgin mating latency in minutes (A), latency to first remating in hours (B) and number of copulations in 24h (C, F) for hearing (\textit{iav}^{1}) , olfaction (\textit{orco}^{1}) , vision (\textit{ninaE}^{17}) and touch (\textit{piezo}^{16}) mutant females raised in social isolation (single) or with social experience (group). Significance stars, or p-value when bordering significance, report condition effect within female genotype tested with mixed effects models. N=18-41. Virgin mating latency in minutes (D), latency to first remating in hours (E) and number of copulations in 24h (F) for females from partitioned vials, illustrated in supplementary figure 1. Significance tested between females from each side of a testing vial. N=7-8. Virgin mating latency in minutes (G), latency to first remating in hours (H) and number of copulations in 24h (I) for \textit{CS} females with arista or third segment of the antenna surgery. Significance stars, or p-value when bordering significance, report condition effect within surgery type tested with general linear models. N=13-15. Virgin mating latency in minutes (J), latency to first remating in hours (K) and number of copulations in 24h (L) for females raised singly, grouped or with fly extract simulating different group sizes. Significance stars reported for conditions differing from single raising tested with general linear models. N=15-20. All females tested with group raised \textit{CS} males. For detailed statistics see supplementary table 1. * p<0.05, ** p<0.001, ***p<0.0001.

Since the effect of immediate social context can be largely explained by olfaction (figure 1) and it was previously suggested that both olfaction and hearing play important roles in determining sexual receptivity in females (Markow, 1987; Rybak et al., 2002), we aimed to study these two sensory modalities in more detail in wild-type flies to study the effect of these sensory modalities in a stable genetic background. To manipulate hearing or olfaction, surgery was performed on \textit{CS} females, cutting either the arista, involved in hearing (Göpfert and Robert, 2001; 2002; Morley et al., 2012), or the third segment of the antenna, harbouring the neurons of classical odorant and ionotropic receptors (Benton et al., 2009). When the aristae are cut, we observe that females need in general more time to accept the virgin mating, but social isolation still slightly shortens this latency, similar to control females (figure 3G). Without the third segment of the antenna (which also eliminates the arista), the virgin latency is significantly increased after social isolation (figure 3G), meaning that olfaction might be necessary for the decrease in virgin latency normally seen. Post-mating receptivity is decreased after social isolation in all groups (figure 3E and 3F), most probably because the females with social experience were transferred to a new environment whereas the socially isolated were kept unchanged, introducing a confounding factor during raising. Nevertheless, the decrease in post-mating receptivity is further exaggerated for females missing the antenna compared to control females (figure 3E and 3F). This experiment gives weak evidence that olfaction and not hearing plays a role in determining female sexual receptivity, both virgin and post-mating. One last line of evidence for the involvement of olfaction comes from a social experience simulation experiment in which \textit{CS} females were raised in isolation, but in the presence of fly pheromone extract from different numbers of flies to simulate different group sizes. In this experiment, only the virgin latency is tentatively affected with a non-significant increased time to virgin mating after exposure to a group and most concentrations of pheromones and a significant increase for the highest concentration of pheromones (figure 3J). Overall, these data suggest that vision, touch and olfaction all play a role in determining early long-term social experience.
Discussion

Social experience modulates reproductive behaviour, a phenomenon that probably evolved as a consequence of living in a group providing benefits and challenges impacting fitness. Reproductive behaviours are, therefore, hypothesized to be modulated. Here, we show that immediate social context (figure 1) and early long-term social experience (figure 2) have opposing effects on female receptivity. Experiencing an increased social context immediately by mating in a group, compared to mating in a single pair, increases female post-mating receptivity (figure 1B and 1C). Whereas early social experience with same-sex individuals, compared to social isolation, decreases both virgin and post-mating receptivity (figure 2A-C). The immediate social context is mediated through the presence of volatile fly pheromones (figure 1H and 1I). Determining long-term experience seems to involve vision, touch and olfaction (figure 3).

Here, olfaction came up as one of the important sensory modalities for both immediate and early social experience with respect to female sexual receptivity. The involvement of olfaction in determining early social experience is observed when the third segment of the antenna is cut (figure 3G-I), thereby removing olfaction including ionotropic receptor neurons (Benton et al., 2009). Additionally, when the social raising environment was simulated with pheromone extract the effect of olfaction was observed (figure 3J-L), but not with females mutant for classical odorant receptors (Orco, figure 3A-C). The lack of effect in classical odorant receptor mutants suggests that olfaction of volatile fly pheromones does not act through odorant receptors, but perhaps ionotropic receptors. Here, these receptors have not been specifically manipulated because of the findings that fly pheromones are often detected through odorant receptors rather than ionotropic receptors (Lebreton et al., 2014; 2017a; van der Goes van Naters and Carlson, 2007). Nevertheless, this hypothesis could be tested through more targeted interventions like the use of ionotropic receptor mutants. Additionally, none of the olfactory or other sensory modality manipulations were sufficient for the effect of early social experience on all three measures of female receptivity. This could mean that females use several sensory modalities to determine social experience, similar to when males respond to early rival exposure with increased mating duration for which the rival detection is determined by at least two modalities (Bretman et al., 2011; Kim et al., 2012b). A valuable experiment to complement this work would thus be to manipulate several sensory modalities at once to further understand how females experience early long-term social exposure.

The usage of several modalities by females to determine early social experience might serve as an insurance, comparable to how females use the integration of hedonic and nutrition value to determine the availability of yeast (Gorter et al., 2016). Responding to one sensory modality can be beneficial when a fast response is needed. With an immediate social context, for example, it is beneficial to quickly exploit the situation and respond back when the context changes. Using one modality is then sufficient and might work as a fast reflex. However, with early long-term exposure, long-lasting changes might occur in the central nervous system due to loss of plasticity after a sensitive period (Golovin and Broadie, 2016).
The finding that long-term experience changes all three measures of receptivity and immediate experience only modulates post-mating receptivity, suggests that long-term experience indeed leads to an overall change in the level of receptiveness. Therefore, the impact of early long-term social experience seems much larger and using several modalities might serve to ascertain the correct perception of context, reducing the chance of a long-lasting maladaptive response.

The involvement of different mechanisms in determining immediate or early long-term social experiences does not explain why these experiences have opposing effects. Female receptivity can be modulated by immediate social context because of the benefits it provides; higher availability of mates, safety of group living and increased changes for offspring survival due to communal egg-laying (Duménil et al., 2016; Etienne et al., 2002; Lin et al., 2015; Lof et al., 2009; Wertheim et al., 2002a; 2002b; Yang et al., 2008). Higher receptivity allows the female to exploit these benefits. However, immediate social context could also modulate female receptivity because of the challenge of competition with other females (Bath et al., 2017). Higher receptivity might then serve to outcompete other females and thus ensure reproduction in conditions where males are scarce. These hypotheses both explain why immediate social context leads to increased female receptivity, but what does early social exposure signal that explains a decrease in future receptivity as compared to social isolation. Or is social isolation simply a stressful situation leading to maladaptive reproductive behaviour?

A first hypothesis for an increase in receptivity after social isolation is that the context experienced early in life prepares a female for the context experienced during reproduction later in life. This suggests that females raised in isolation also perform better in single pair mating and vice versa. If the level of receptivity is a read-out of performance, a match-mismatch experiment could show whether this is true. In this type of experiments, females raised in groups or in isolation are then tested in a single pair context and a group context, providing a full factorial match or a mismatch between early and immediate social experience. Here, we are missing the comparison of females raised in isolation and tested in a group context, but females raised in isolation and tested in a single pair increase sexual receptivity (figure 2A-C). Additionally, females raised in a group increase receptivity when they are tested in a group as compared to a single pair (figure 1B and 1C). However, literature suggests that females raised in isolation increase receptivity even further when tested in a group context (Ellis and Kessler, 1975). This does not support a match-mismatch theory where the level of receptivity is the measurement of performance. However, receptivity might not be so directly translated into performance as this suggests an increase in fitness, which is an equation of lifespan and reproduction. Preliminary results on lifespan and reproduction, indicate that the matched condition of raised in isolation and tested in single pairs does not lead to higher fecundity as compared to the mismatched condition of raised in a group and tested in single pairs (supplementary figure 2A-D) nor an advantage or disadvantage in lifespan (supplementary figure 2E and 2F). Increased sexual receptivity after long-term social isolation, therefore, does not seem to serve as a preparation for the future context.
Alternatively, early long-term social isolation might lead to an overall heightened state of arousal for both females and males (Ellis and Kessler, 1975; Goncharova et al., 2016). Social experience during early life can lead to learned choosiness; social isolation is a lack thereof. Male *D. melanogaster* raised with same-sex individuals, for example, show negative-conditioning in courtship due to recurring rejection (Bastock and Manning, 1955) and they increase competitive behaviours after long-term exposure to rivals (Bretman et al., 2011; Goncharova et al., 2016; Kim et al., 2012b; Liu et al., 2011). This hypothesis suggests that not learning this behaviour due to social isolation should lead to sexual activity towards inappropriate subjects. Indeed, this is illustrated by a decrease in sexual isolation between six subspecies of *Drosophila paulistorum* after early long-term isolation (Kim et al., 1992) and overall increased sexual activity (Kim and Ehrman, 1998). Here, we see increased sexual receptivity in females as well and females also accept poor quality males (y,w) faster after social isolation, which might suggest a lack of choosiness.

Another cause for a heightened state of arousal and lowered level of choosiness could be an increased sensitivity to sexual signals from conspecifics leading to quicker acceptance of a potential mate sending the right signals. The lack of social experience signals low density and might increase the sensitivity to signals of potential mates leading to increased sexual receptivity to ensure reproduction when the opportunity presents itself. In this situation, the flies cannot afford to be too choosy and should thus mate with any appropriate mate. In this theory, high receptivity is adaptive to make the best of a bad situation. An increase in pheromone production in both *D. melanogaster* and *D. paulistorum* after social isolation (Farine et al., 2012; Kim et al., 2004) might support this theory as it could serve to actively attract conspecifics. However, for females, we found that isolated individuals were not more attractive to courting males than group raised females, but they could still be better able in attracting flies from longer distances. Whether increased sexual receptivity after social isolation is an adaptive behaviour is uncertain at this point; it does not seem to be beneficial in itself, but it could be a means of making a bad situation work. Whereas, mating in a group seems highly recommended.

**Authors and Contributors**

J.A.G. performed and supervised all the experiments included in the manuscript. J.A.G. performed all statistical analyses. J.-C.B. and J.A.G. designed the experiments and wrote the manuscript.
Acknowledgements

We thank A. Sarma for performing a subset of experiments on sensory modalities and lifespan; A. Ebskamp and M. Luxwolda for performing a subset of experiments on sensory modalities; L. Kauffmann, D. Meijerink and N.F. Peen for performing experiments on female attraction; G.J.F. Overkamp for counting offspring data; H. Doornbosch for setting up a subset of experiments on sensory modalities; E. van Es for her help with performing antenna and arista surgery, setting up experiments and analyses. We are grateful for A. Soto Padilla for reading the manuscript. This project was funded by a Neuroscience Research School BCN/NWO Graduate Program grant (ref 022.OO4.OO8).
**Supplementary Information**

**Supplementary Figure 1: Simulating social experience** Picture of raising context with one female (clear single) separated from a group of six (clear group) with a clear partition in a standard food vial (A). Picture of raising context with one female (dark single) separated from a group of six (dark group) with a dark partition in a standard food vial (B). Picture of raising context with one female (Net single) separated from a group of six (Net group) or empty space (Net iso) with a clear partition and netted top rail to allow transfer of sound and smell (C).
Supplementary Figure 2: Fitness consequences of social experience Number of eggs laid during the 24h of the experiment (A), index of number of offspring hatched from the eggs laid during the 24h of the experiment (B), lifetime number of offspring per female (C), number of offspring hatched until each day of transfer (D), average lifespan in days (E), survival curve (F) for single or group raised C5 females tested with C5 males. Significance stars or p-value reported of condition effect tested with linear model. N=20.
### Chapter 4

#### Table 1: Summary statistical analysis

All models in this table were performed using R version 3.2.2. All data that complied with the rules of normality and homogeneity were tested with (mixed effects) linear models and the output is shown here with t- and p-values. When either normality or homogeneity could not be satisfied the data were log transformed, sometimes followed by normalization. When applicable a random effect for date was added to the models.

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#### Figure 2: Fitness consequences of social experience

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*Log likelihood ratio test  **Test statistics (contingency table convert)