General introduction

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Introduction

Sexual reproduction requires cooperation between a male and a female to produce viable offspring. The likelihood to engage in reproduction differs per individual based on genetic background, which leads, for example, to different levels of sexual activity or sensitivity to cues from the other sex. The environment the pair meets in also impacts the individuals' likelihood of reproduction. An environment with high nutritional quality is a good place for reproduction due to available resources for egg production and offspring rearing. In addition to food, social interactions beyond the pair can facilitate reproduction, since living in a group reduces the individual risk of predation, increases offspring survival and provides higher mate choice. Therefore, individuals have likely evolved mechanisms to couple sex drive to the environment. Lastly, prior experiences of each partner might modulate their sexual activity. For example, after many rejections males might reduce investment in courtship displays, whereas with prior successful mating experience a female might be less receptive to mate again. Reproduction is thus not only an interaction between a male and a female influenced by their genomes, but is also majorly impacted by their environment and prior experience.

Sexual reproduction

Different organisms have different mating systems; a description of the way sexual interactions are structured around reproduction. Mating systems include monogamy (partner bond between one male and one female) and polygamy (several mating partners per individual). Polygamous mating systems are polygyny (partner bond between one male and several females), polyandry (partner bond between one female and several males), polygynandry (both sexes have several partners) and promiscuity (no partner bond, frequent different partners, often implying indiscriminate mate choice). Males are commonly seen as the promiscuous sex and females are depicted as choosy (Bateman, 1948). This is for example illustrated by the higher occurrence of polygynous pair bonds in human populations than polyandrous pair bonds (Archetti, 2013). However, development in paternity testing is revealing that female promiscuity, or polyandry, is more widespread than previously thought in the animal kingdom and might be the rule rather than the exception (Holman and Kokko, 2013; Parker and Birkhead, 2013; Taylor et al., 2014). The same realisation is starting to emerge in the model organism *Drosophila melanogaster*. For a long time, female fruit flies were thought to accept a low number of mates and go for long periods of time without mating, but it is becoming clear that *D. melanogaster* is a promiscuous species in which both sexes frequently mate with different individuals within short timeframes (Giardina et al., 2017; Imhof et al., 1998; Ochando et al., 1996).

Drosophila melanogaster male courtship and female receptivity

The mating of *Drosophila melanogaster* consists of males performing an elaborate courtship sequence towards females (Spieth, 1974). This male display consists of the following behaviours: orienting, tapping, following, wing vibration, licking genitalia, mounting and copulation attempt ((Bastock and Manning, 1955; Spieth, 1974), illustrated in figure 1). When subjected to male courtship, females can accept or reject the males' interest with rejection signals (Hall, 1994). Rejection is signalled through wing fluttering, decamping, fending, kicking and full ovipositor extrusion (Bastock and Manning, 1955; Dukas and Scott, 2015; Hall, 1994; Markow and Hanson, 1981). Receptivity to mating is signalled by the females slowing down (Bussell et al., 2014; Fabre et al., 2012; Markow and Hanson, 1981; Tompkins et al., 1982) and increased abdominal preening, partial ovipositor extrusion and droplet emission, which have been suggested to function to spread a chemical cue signalling willingness to the mate (Lasbleiz et al., 2006). Next, females spread their wings to allow males to mount and finally open the vaginal plates to accept a copulation attempt (Bastock and Manning, 1955). After a copulation attempt is accepted and mating has taken place, the male and female go their separate ways.

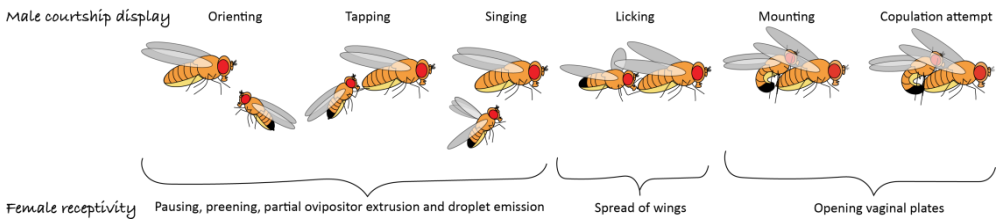

Figure 1: Male courtship and female receptivity Schematic overview of the different components of the male courtship sequence and the observed female cues of receptiveness. The first steps in the male courtship sequence are often repeated several times, before the first female cues of receptiveness start to appear.

Sexual conflict over female multiple mating

After a first successful mating, both sexes can, in theory, move on to the next sexual partner. However, when females remate this diminishes the chances of fertilisation for the first male (Bateman, 1948; Lefevre and Jonsson, 1962) as the second male's sperm fertilises about 80% of the offspring (Clark et al., 1995). Males have evolved countermeasures to increase their individual reproductive success. The first of such countermeasures is the adjustments of the ejaculate size when a male senses rival males in his environment (Garbaczewska et al., 2013; Sirot et al., 2011; Wigby et al., 2009). Since the sperm of different males compete within the female reproductive tract for fertilisation (Lefevre and Jonsson, 1962; Manier et al., 2010; Parker and Pizzari, 2010), males with bigger ejaculates might gain an advantage as they transfer more sperm to compete with that of other males (Letsinger and Gromko, 1985). Not only the size, but also the composition of the ejaculate is adjusted in the presence of competitor males (Wigby et al., 2009). By increasing the proportion of peptides that decrease

female receptivity like Sex peptide (Sp,(Chapman et al., 2003; Chen et al., 1988; Liu and Kubli, 2003)), discussed in more detail in section "A decrease in female receptivity after mating", males might decrease the chances of being outcompeted by consecutive males. Another form of countermeasure is mate guarding, chemically or physically. Chemical mate guarding is achieved by transferring pheromones, such as cis-Vaccenyl Acetate (cVA) and 7-Tricosene (7-T), that make mated females less attractive to other males (Jallon, 1984; Laturney and Billeter, 2016; Yew et al., 2009; Zawistowski and Rollin, 1986). Physical mate guarding can be observed as a mating plug that might make it physically more difficult to remate, as it does in butterflies (Kawahara et al., 2017). More importantly, the mating plug in *D. melanogaster* serves to assure sperm retention (Avila et al., 2015; Lung and Wolfner, 2001) and contains compounds that make females less attractive (Guiraudie-Capraz et al., 2007) and less receptive (Bretman et al., 2010).

These male adaptations make it more difficult for a female to gain consecutive matings. Females have, however, evolved countermeasures to regain control over reproduction. For example, females eject the mating plug, part of the ejaculate and the associated pheromones and possibly peptides (Laturney and Billeter, 2016; Lee et al., 2015; Lüpold, 2013; Manier et al., 2010), recovering her attractiveness and making her more accessible for following mates. These illustrations show that male and female *D. melanogaster* are in sexual conflict over the level of female receptivity after the first mating (post-mating receptivity).

Female multiple mating is determined by her receptivity

Why females remate with several males is a controversial topic. For a virgin female the reason to mate is intuitive, it serves to ensure offspring production and therefore her fitness (Kokko and Mappes, 2005). Additionally, wild-caught virgin *D. melanogaster* females have a lower lifespan than mated ones, so mating might even provide direct health benefits or alternatively there might be a cost of staying virgin (Markow, 2011). Since female fitness is not constrained by sperm availability (a single male ejaculate has more sperm than a female has eggs), but by egg production, a female does not "need" to remate for several days until her sperm storage are exhausted (Bateman, 1948; Lefevre and Jonsson, 1962). Additionally, several costs to mating have been documented. Mated females suffer decreased immunity caused by male seminal fluid peptides (Chapman et al., 1995; Fedorka et al., 2007; Schwenke and Lazzaro, 2017; Short et al., 2012), physical harm (Kamimura, 2007) and decreased lifespan and lifetime fecundity (Kuijper et al., 2006). However, multiple matings have also been proposed to serve different benefits like ensuring fecundity, producing genetically diverse offspring (Billeter et al., 2012; Jennions and Petrie, 2000), trading up for a better quality or adapted male (Bleu et al., 2012; Jennions and Petrie, 2000; Long et al., 2010; Seeley and Dukas, 2011) and to receive seminal peptides that increase egg production and the fitness of the offspring (Fricke et al., 2010; Herndon and Wolfner, 1995; Priest et al., 2008). Another reason for remating might be "convenience polyandry", whereby a female does not

remate to gain direct benefits, but to reduce harassment by males as mating typically reduces this for a short time after mating (Newport and Gromko, 1984; Rowe, 1992).

Taking the costs and benefits into account, it is suggested that females should evolve an intermediate copulation number to balance the costs and benefits and maximise their fitness (Arnqvist and Nilsson, 2000). However, number of copulations is not a direct female trait and females cannot be expected to predict or keep track of the number of mate encounters (Kokko and Mappes, 2012). Therefore, selection must act on a female sexual trait that can indirectly determine the number of copulations. This trait is suggested to be a female's acceptance threshold or level of receptivity (Kokko and Mappes, 2012). When a female has a high level of receptivity, she is more likely to accept than reject a male each time she encounters one and, therefore, will end up with a higher number of copulations during her lifetime than the suggested intermediate number (Kokko and Mappes, 2012). This theory provides an explanation of why females mate more often than necessary to maximise fitness, but it assumes a fixed level of receptivity over a female's lifetime (Kokko and Mappes, 2012). However, the level of receptivity is known to be plastic and influenced by other factors like a female's mating state (Kokko and Mappes, 2005), with mated females displaying lower levels of receptivity than virgin females. Female mating is thus instructed by her sexual receptivity and the level of receptivity can be adapted to mating state.

Neuronal circuitry of virgin receptivity

Research on the mechanisms underlying a virgin female's sexual receptivity has revealed sensory pathways involved in detecting and processing different male courtship signals. The most important male sexual signal for a virgin female appears to be the quality of the courtship song (Dickson, 2008; Markow, 1987; Rybak et al., 2002). A second main stimulating medium for females during courtship are pheromones (Billeter et al., 2009). The identified elements of the neuronal circuitry regulating virgin receptivity are based on sensing and responding to these courtship signals. Females express an odorant receptor called *Or67d* (Bartelt et al., 1985; Kurtovic et al., 2007) that detects a stimulatory male pheromone, 11-cis-vaccenyl acetate (cVA) (Bartelt et al., 1985; Kurtovic et al., 2007). Females lacking this receptor are less receptive than wild-type females (Kurtovic et al., 2007). Further sensory processing of this pheromone as well as the courtship song is achieved by neuron clusters in the posterior dorsal protocerebrum in the central brain that gets activated when female are exposed to these male signals (Zhou et al., 2014). Inactivation of these neurons via tetanus toxin expression leads to decreased copulation by females (Zhou et al., 2014). After detection of male cues, receptive females show pausing behaviour preceding a copulation (Bussell et al., 2014). This pausing behaviour is coordinated by *Apterous*-expressing neurons in the brain (Aranha et al., 2017; Ringo et al., 1991) and *abdominal-B*-expressing neurons in the abdominal ganglion, ventral nerve cord and reproductive tract. Together, these accounts show that the main mechanism for virgin receptivity is based on how females determine male

courtship cues and show acceptance behaviour, suggesting increased receptivity in direct response to a male encounter and little involvement of any other factors like environmental richness.

A decrease in female receptivity after mating

Females undergo behavioural changes after mating known as the post-mating response, which results in changes in diet preference (Ribeiro and Dickson, 2010), increased oviposition (Heifetz et al., 2005; Herndon and Wolfner, 1995; Ram and Wolfner, 2007) and decreased receptivity towards courting males (Manning, 1967). Females signal this decrease in receptivity to males with full ovipositor extrusion, a rejection behaviour exclusive to mated females (Manning, 1967). The change in receptivity has been subdivided into a short-term or "copulation" effect, from directly after mating up to 48h, and a long-term or "sperm" effect, from 24h to 10 days after mating (Manning, 1967).

The short-term effect has been linked to substances produced and secreted by the accessory glands of *Drosophila* males (Chen et al., 1988; Kalb et al., 1993). Females mated with males missing the main accessory gland cells show increased remating at 24h as compared to mated with wild-type males (Kalb et al., 1993), accessary gland peptides must thus be involved in the reduction of receptivity shortly after mating. Among those peptides is Sex peptide (Sp), which has the ability to decrease remating demonstrated by artificial injection in the abdominal cavity or ectopic expression by means of transgenes in females (Aigaki et al., 1991; Chen et al., 1988). Furthermore, Sp has been weakly implicated in the short-term response at 24h (Chapman et al., 2003; Liu and Kubli, 2003). However, due to this weak response, it is debated whether Sp is the actor of the short-term decrease in receptivity. Another factor able to elicit a short-term decrease by artificial injection into the female abdomen is the ejaculatory duct peptide DUB99B (Saudan et al., 2002). Whether this peptide is actually involved in the short-term effect is unclear as accounts of females mated with males that lack accessory glands, but still produce normal amounts of DUB99B, do not show the characteristic decrease in post-mating receptivity, suggesting not only that DUB99B is not sufficient but also that accessory gland proteins are necessary for this effect (Rexhepaj et al., 2003; Xue and Noll, 2000). A third factor involved in the short-term decrease of receptivity is the mating plug protein PEBII produced in the ejaculatory bulb, which directly decreases female receptivity within the first 4h after mating (Bretman et al., 2010). Even though PEBII is a mating plug protein and mutant males produce smaller plugs, this does not lead to changes in fecundity (Bretman et al., 2010). This suggest that the effect of PEBII is not due to its function as mating plug protein, but it might work by acting on the female receptivity pathway.

For the long-term response of mating on female receptivity, both sperm and the accessory gland peptide Sp are necessary (Kalb et al., 1993; Peng et al., 2005). Sp elicits a reduction in female receptivity after ectopic expression or artificial injection (Aigaki et al., 1991; Chen et al., 1988), but the proof of Sp's involvement in the long-term effect comes from knockdown studies with RNA interference and genetic mutations where males without

Sp are unable to elicit a long-term decrease in female receptivity (Chapman et al., 2003; Liu and Kubli, 2003). For the long-term effect in receptivity reduction, Sp requires association with sperm (Kalb et al., 1993). Sp is bound to and gradually released from sperm, due to cleavage at the N-terminal end of the peptide (Peng et al., 2005). To assure this effect of Sp, the picture is more complex with several other male accessory gland peptides involved which, for example, stabilize the Sp-sperm bond, ensure proper transfer of sperm and localisation into the sperm storage organs (Ram and Wolfner, 2007; 2009; Sirot et al., 2009; Sitnik et al., 2016).

The effect of Sp on female receptivity is mediated by the G-protein coupled Sex peptide receptor (SPR), located in the female reproductive tract as well as throughout the central nervous system (Yapici et al., 2008). A small number of SPR sensory neurons on each side of the uterus are necessary for sensing Sp and to elicit a decrease in female receptivity (Häsemeyer et al., 2009; Yang et al., 2009). These neurons relay information onto neurons in the abdominal ganglion (SAG neurons, (Feng et al., 2014; Rezával et al., 2012)), which in turn project to the dorsal protocerebrum in the central nervous system which is an area known to be involved in female receptivity (Feng et al., 2014). Inhibition of the SAG neurons by Sp sensory neurons signals mated state to the protocerebrum and decreases female receptivity, while hyperactivation leads to virgin-like female receptivity (Feng et al., 2014). Additionally, the post-mating response can be induced by Sp acting directly on neurons in the central nervous system in the absence of SPR, but only with ectopic neuronal expression of Sp or a leaky blood-brain-barrier (Haussmann et al., 2013). This suggest that Sp also acts directly on the central nervous system, bypassing the SPR pathway.

Reduced female receptivity after mating is not final, fixed or general. Females can counteract these effects by ejecting the mating plug including the short-term factors as well as the ejaculate, potentially including a portion of the factors for the long-term effect (Laturney and Billeter, 2016; Lee et al., 2015; Manier et al., 2010). Sperm ejection can therefore restore females' ability to remate. Next to that, not all strains of *D. melanogaster* show the same level of post-mating response, some strains, like wild-type *Canton-S*, stay more receptive, both short- and long-term, even though they receive the same factors as less receptive strains (Denis et al., 2017).

Mechanisms of post-mating receptivity, when it does occur

Beyond the effect of male peptides inducing decreases in receptivity as discussed above, the mechanisms and modulators of female post-mating receptivity are not well understood. One potential contributing factor to this incertitude are the type of behavioural assays employed by the field that studies it. Female post-mating receptivity has mostly been explored using an assay in which the female is provided with an initial opportunity to mate, followed by opportunities to remate every 24h or 48h for a fixed amount of time (30 min- 2h). In between mating sessions, the female is isolated on egg laying substrate. This confinement assay has revealed that female post-mating receptivity, quantified as the time to remating (in days) or

the likelihood of remating at a certain time point, has a genetic basis that can be selected for (Fukui and Gromko, 1991a; 1991b; 1991c; Gromko and Newport, 1988a; 1988b; Pyle and Gromko, 1981) and that sperm presence in the female sperm storage organs is a main determinant of remating (Letsinger and Gromko, 1985; Newport and Gromko, 1984). Females that receive more sperm are less likely to remate and take longer if they do and, similarly, females mated with sperm-depleted males are more willing to remate (Lefevre and Jonsson, 1962). In accordance, studies investigating the genetics underlying post-mating receptivity have identified genes associated with sperm storage and immunity, which is also affected by male seminal peptides, as well as odorant binding proteins possibly facilitating interactions between males and females (Giardina et al., 2011; Lawniczak and Begun, 2004). This confinement assay, therefore, shows an effect of sperm presence in female storage and suggests that female receptivity is more reflective of a reflex to substances transferred by the male rather than a malleable response.

However, the effect of sperm presence can be modulated and may be a result specifically observed in the confinement assay. For example, the availability of food for females in this assay can increase her willingness to remate even before her sperm storage is depleted (Harshman et al., 1988). Furthermore, this assay suggests that females rarely remate or at least take several days to do so, which is in conflict with the finding that females in the wild mate at least once per day (Giardina et al., 2017) and often carry sperm from several males (Imhof et al., 1998; Ochando et al., 1996). Other assays have, therefore, tried to mimic natural variation by continuously housing mating pairs for longer periods of time, up to 48h (Krupp et al., 2008; 2013; Lefevre and Jonsson, 1962; Newport and Gromko, 1984; Smith et al., 2017; van Vianen and Bijlsma, 1993). In such continuous assays, females remate more often with an average of up to 6 times per 24h depending on the strain and context (Billeter et al., 2012; Krupp et al., 2008; 2013). Remating in this assay does not depend on the amount of sperm left in female storage (Newport and Gromko, 1984). The continuous assay has been suggested to result in more harassment for the female and less opportunity to decamp, and thus reject the male (Newport and Gromko, 1984). This is used to explain why a higher post-mating receptivity is observed compared to the confinement assay. However, 50 percent of females remate within 6h after the first mating (Smith et al., 2017; van Vianen and Bijlsma, 1993) both when continuously housed with a second male and when the second male is introduced 3h later (van Vianen and Bijlsma, 1993), suggesting that the effect is due to an internal change in receptivity of the female rather than continuous inability to reject the male. Additionally, the level of post-mating receptivity can be largely explained by female rather than male genotype, as 47 per cent of the variance in post-mating frequency depends on the strain of the female and only 11 on that of the male (Billeter et al., 2012), giving difference in male courtship and seminal peptides less impact than differences in female willingness. Thus, post-mating receptivity depends on female genetics and different factors can affect it depending on context. What the genetic differences and modulators are that determine mated female receptivity are, however, still largely unidentified.

Environmental influences on female receptivity

Since high receptivity is a costly phenotype due to resource allocation towards egg-production as well as the costs incurred during mating, female sexual receptivity is likely to be influenced by environmental factors. Females might increase receptivity when they receive cues that resources are plentiful. Food is a likely candidate to influence receptivity as females are dependent on this external resource to produce eggs and viable offspring (Becher et al., 2012; Bownes et al., 1988; Lee et al., 2008; Terashima, 2004). Indeed, the availability of food (determined in a confinement assay) influences post-mating, but not virgin, receptivity (Harshman et al., 1988). The modulation of receptivity connected to food availability only occurs when females still have sperm in storage. Female receptivity increases by sperm-depletion and this is not further affected by food availability (Harshman et al., 1988). Additionally, females fed on high nutritional diets, protein levels or overall food content, increase remating measured as time to remating in days or occurrence of remating over the whole lifespan with daily exposure (Chapman and Partridge, 1996; Schultzhaus and Carney, 2017). Food can thus modulate female receptivity, but the mechanisms underlying this modulation of receptivity are unknown.

Another factor that influences female receptivity is social context. There are several reasons why females can be expected to increase sexual receptivity in a dense social context, an environment with many individuals of the same species. The first reasons is that there are more males for the female to choose from providing the possibility to produce more diverse offspring (Billeter et al., 2012; Jennions and Petrie, 2000). Second, females prefer to lay their eggs communally (Duménil et al., 2016; Lin et al., 2015; Lof et al., 2009; Wertheim et al., 2002a; Yang et al., 2008), because higher density of adults and larvae keeps the fungal growth to a minimum which would otherwise decrease larval development (Wertheim et al., 2002b). Third, a female might experience competition with other females for egg laying substrate or available mates, especially after mating, which could be reflected in an increase in aggression as well as higher receptivity to increase her chances of getting the best mates. Indeed, it is shown that females increase aggression towards other females after mating (Bath et al., 2017), but whether this is further increased in higher density is unclear. In regards to sexual receptivity, females have indeed achieve mating faster when tested in big group as opposed to single pairs (Ellis and Kessler, 1975; Laturney and Billeter, 2016), post-mating receptivity can increase in response to higher density (Harshman et al., 1988) and females caught inside a high density winery have a higher paternity estimations as opposed to low density woodlands outside (Marks et al., 1988). Lastly, more genetic diversity in the social context during mating increases the number of copulations in 24h of mature females, which is blocked when females are unable to sense their environment through classical odorant receptors (Billeter et al., 2012; Krupp et al., 2008). However, how social density instructs female receptivity is still to be determined.

Is post-mating receptivity a return to virgin receptivity?

It is clear that virgin females are much more sexually receptive than mated females. Mated female receptivity is often interpreted as the return of virgin-like state after several days (Kalb et al., 1993; Peng et al., 2005), but is it the same receptivity? Theory predicts that virgin and mated females should not use the same rules when it comes to receptivity due to a different balance in the cost-benefit of mating as previously discussed (Arnqvist and Nilsson, 2000; Jennions and Petrie, 2000; Laturney and Billeter, 2014). However, they could use the same mechanisms, but perhaps with emphasize on different cues.

For virgin receptivity, most neuronal pathways identified are involved in the response a female shows towards the male's courtship advances (Aranha et al., 2017; Bussell et al., 2014; Kurtovic et al., 2007; Zhou et al., 2014). Even though a mated female similarly responds to the courtship signals (song and cVA) and decreases movement before mating, some of the accounts on virgin receptivity show that the same manipulations do not impact post-mating receptivity, measured as mated female full ovipositor extrusion (Aranha et al., 2017; Bussell et al., 2014). This lack of effect of the virgin mechanism manipulations on post-mating receptivity suggests that even though mated females signal receptivity in a similar manner, this behaviour might be invoked differently in virgin versus mated females. Therefore, virgin and post-mating receptivity might not share the same mechanisms. Most of the known mechanisms of mated female receptivity are involved in sensing or dealing with male compounds transferred during mating (Chapman et al., 2003; Letsinger and Gromko, 1985; Liu and Kubli, 2003), which is a specific challenge for mated females. However, this challenge could be a new factor feeding into the same mechanisms for receptivity and, therefore, does not provide any indication whether virgin and mated females use the same mechanisms.

Reviewing studies that have both reported virgin and post-mating receptivity suggests that these two processes rely on different mechanisms. First, selection experiments based on female remating speed (assessed with a confinement assay) show that the resulting virgin mating latency is either uncorrelated, positively or negatively correlated with remating latency (Gromko and Newport, 1988b; Pyle and Gromko, 1981). This shows that genetic selection for fast post-mating receptivity does not select for either fast or slow virgin receptivity suggesting different genetic architectures for virgin and mated female receptivity. Second, an account of female mating in a continuous assay also shows that time to first remating is uncorrelated with virgin mating latency (van Vianen and Bijlsma, 1993). Third, an attempt to correlate variation in 10 candidate genes to variation in several measures of female sexual behaviour, including mating latency and remating, finds variation in some of the same genes correlated to both virgin and post-mating receptivity, but never the exact same variation site (Giardina et al., 2011). These examples suggest a different genetic background for the two behaviours.

Research has focussed on mechanisms of female receptivity as virgins, the switch between virgin to mated state facilitated by Sp and what factors explain an earlier return of

receptivity in mated females. However, post-mating receptivity might not be the return of virgin receptivity, but rather a different phenomenon.

Thesis overview

Females are more promiscuous than was previously assumed which suggests that female receptivity is a more nuanced behaviour than a simple on-or-off state where virgin females are receptive and mated females are not. Female receptivity starts with mature virgin females with their specific "decision" rules about whom to mate with and setting their level of receptivity. After a first mating the females' receptivity levels change due to the post-mating response. As mated females are expected to have different costs and benefits determining their receptivity, this predicts that post-mating receptivity is a different trait than virgin receptivity. Here, my main aim is to understand what factors influence female virgin and post-mating receptivity and how these modulators are signalled and sensed. To investigate these factors, I used a continuous mating assay as it more closely mimics the natural levels of female receptivity and allows for continuous manipulation of the environment in which both virgin (latency to virgin mating) and post-mating (latency to first remating and number of copulations in 24h) receptivity are assessed in one assay. The environmental manipulations are achieved by methods described in **chapter 2**, including the use of an airpump system to supply specific odours and manipulation of the components present in the food substrate or mating area to quantify the effect on female mating behaviour.

First, I focus on the influence of environment on female receptivity. The effect of food, which specific nutrient, and how these are sensed to influence female receptivity are tested in **chapter 3**. For testing nutritional cues, the airpump system mentioned above is used as well as different compositions of the food substrates. To determine how specific food components are sensed, genetic mutants for sensory modalities and knockdown of sensory neurons by use of the Gal4-UAS system (explained in box 1) are tested in the different environmental conditions. Next, a similar approach is taken for the social environment in **chapter 4**. The social environment is manipulated by testing different group sizes and the airpump system is employed to determine which signals are necessary to sense group size affecting female receptivity. Additionally, female receptivity is tested after manipulation of the social raising environment as experience can modulate sexual behaviours. These chapters thus cover extrinsic cues involved in female sexual receptivity.

Second, several intrinsic cues of sexual receptivity are investigated. A candidate gene approach is taken for odorant receptors detecting fly odours, namely *Or47b* and *Or88a*, in **chapter 5**. These two candidate genes are explored through genetic mutants and manipulation of the cells these odorant receptors are normally expressed in through the Gal4-UAS system. For the brains of one specific Gal4-UAS manipulation, the olfactory regions associated to these odorant receptor neurons are analysed with immunohistochemistry and volumetric analysis. Last, in **chapter 6**, a genome wide association (GWA) approach is taken to identify new candidate genes and tissues of interest for both virgin and mated female

receptivity. For mated female receptivity a follow-up RNA interference study is performed for a subset of identified genes by use of the Gal4-UAS system. This covers some of the intrinsic factors involved in female receptivity. Altogether, this thesis provides new insights and areas for further exploration of both the nurture and nature of female sexual receptivity.

Box 1: Gal4-UAS binary system

In this thesis, the involvement of specific genes and tissues are questioned for their influence on female sexual receptivity. Next to mutations in the genes of interest, a binary system is used for targeted manipulation. This binary system is the Gal4-UAS system (Brand and Perrimon, 1993). Genetically modified fly stocks are generated to express a yeast (*Saccaromyces cerevisiae*) derived transcription activator protein, Gal4. Gal4 is controlled by a *D. melanogaster* promotor, also known as a driver, to ensure that Gal4 is only expressed in tissue in which this driver is activated. Gal4 drivers can have wide expression patterns, by use of a promotor region of a protein expressed in all neuronal tissue for example, or they can have very specified expression, in cells only expressing a very specific protein. On its own Gal4 has little effect in *D. melanogaster* tissue as it is not endogenous. One of the main targets of Gal4 is a cis-regulatory site (DNA sequence) called Upstream Activating Sequence (UAS). When Gal4 binds to this regulatory site, the DNA sequence UAS regulates can be transcribed. A second set of genetically modified fly stocks, therefore, have an UAS site inserted into the genome followed by a transgene. This transgene can be any genetic sequence ranging from reporter proteins to proteins that manipulate the electric activity of neurons. As for Gal4, on its own the UAS transgene has little effect as it is not recognized by endogenous *D. melanogaster* transcription activators. Then, to manipulate or visualize target tissue, the appropriate Gal4 line is crossed to the UAS line that serves the manipulation's purpose. The offspring of this cross has both transgenes in its genome and the UAS transgene is expressed in the tissue of interest. Here, this system is used to manipulate the activity and development of odorant receptor tissue, to localize rescue of mutations to specific cells and to inhibit candidate gene expression with RNA interference in all brain tissue or a specific brain area. Female offspring harbouring both transgenes are tested for their sexual receptivity as well as, for some specific hypotheses, male offspring for their sexual activity.

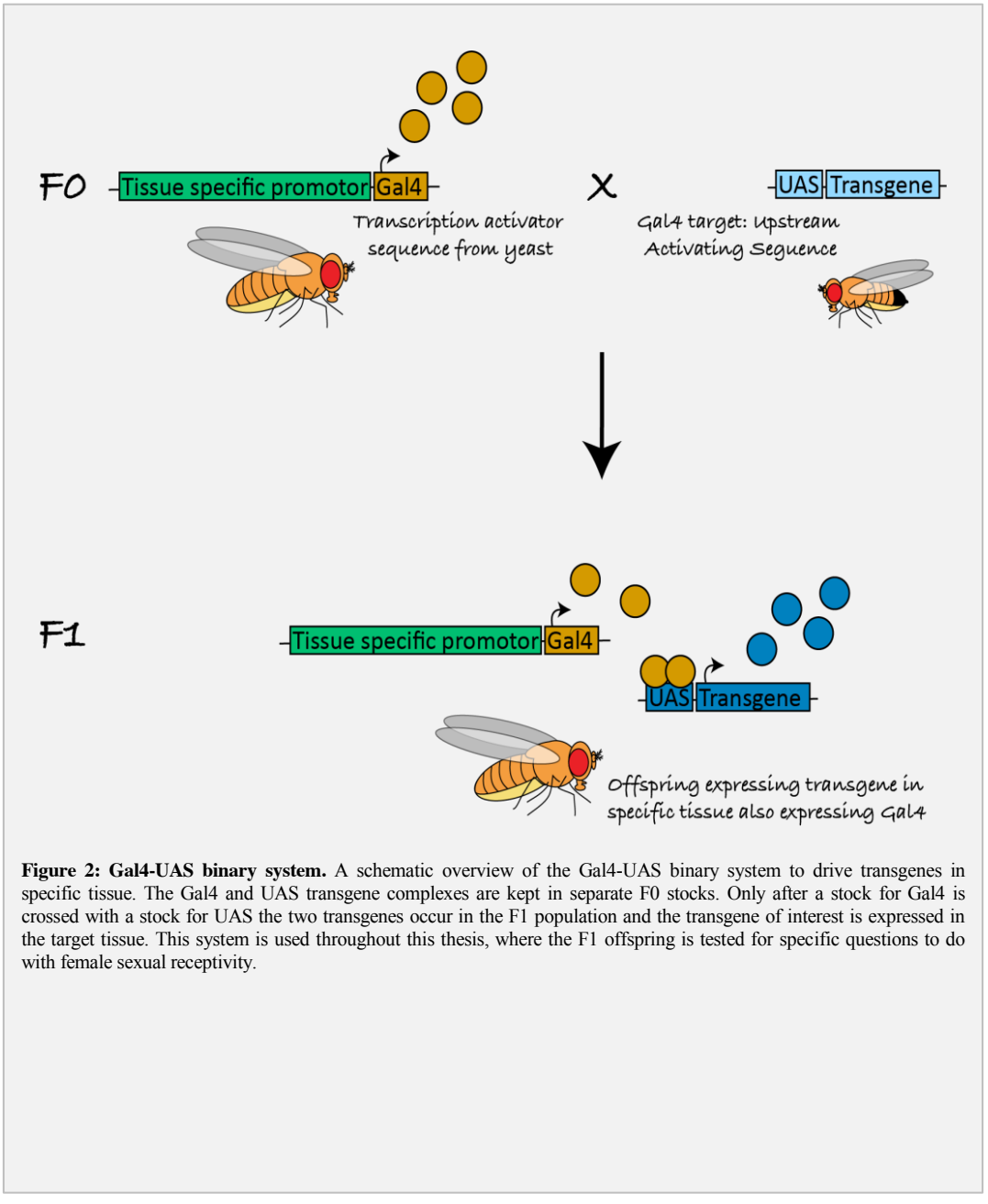

Figure 2: Gal4-UAS binary system. A schematic overview of the Gal4-UAS binary system to drive transgenes in specific tissue. The Gal4 and UAS transgene complexes are kept in separate F0 stocks. Only after a stock for Gal4 is crossed with a stock for UAS the two transgenes occur in the F1 population and the transgene of interest is expressed in the target tissue. This system is used throughout this thesis, where the F1 offspring is tested for specific questions to do with female sexual receptivity.