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Life with others

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Chapter 1

General introduction

Tiphaine P. M. Bailly

Life in groups: the eco-evolutionary significance of aggregative behaviour

Group formation, also called aggregation, is probably one of the most common features of life (Frank, 2007), occurring in most organisms, from bacteria to humans. For example, bacteria aggregate in biofilms (West et al., 2007), social insects live in complex nests (Sokolowski, 2010), large herbivorous mammals form herds, and humans gather in tribes (Parrish, 1999). Why do organisms aggregate? Group living intensifies several opposing forces between group members (Frank, 2007): conflict, for instance over shared resources; cooperation, enhancing individual efficiency and aiding in defence against predators or in competition against other groups; the risk or spread of infectious diseases. The decision to join or avoid others can be affected by the physiological and informational state of the individual and by the contextual state of resource availability and quality. In turn, being in a group can affect the behaviour (e.g. time allocation to feeding), morphology (e.g. size change, dominance characteristics) and physiology (e.g. hormonal changes) of individuals.

Benefits and costs of living in group

A variety of costs and benefits may apply to an aggregation state (Parrish, 1999; Pulliam and Caraco, 1984; Wertheim et al., 2005). Optimal group size is predicted from the benefits and costs to individual members for group membership (Parrish, 1999). Individuals will do what is best for them and seek to maximize their reproductive output and survival. Group forming is selected when individuals who live in aggregates have increased fitness over solitary individuals (Frank, 2011; Krause et al., 2002; Majolo and Huang, 2018). Fitness is a measure that describes the total number of fertile offspring that an individual produces and that survive until reproductive age; it is affected by traits such as the rate of offspring development, the number or size of the offspring, the offspring survival probability, the numbers of mates in a lifetime, and the lifetime reproductive success. As measuring the total number of fertile and surviving offspring is difficult, a selection of these traits that contribute to life time reproductive success are often measured as proxy for fitness. Each trait in nature that affects fitness and is heritable is shaped by natural selection towards an optimal balance between benefits and costs. Below are some examples of the benefits and costs for individual fitness for living in groups.

Benefits

1- Mate finding: Being in a group can increase fitness simply by facilitating mate finding. The arrival of the breeding season triggers aggregation for several otherwise solitary animals. For instance, frogs abandon their solitary hibernation sites for communal breeding places in shallow ponds (Cummins, 1920), and water isopods stop free floating during spring

and swim counter current to seize other isopods and form a compact and stable cluster that facilitates mate finding (Allee, 1927).

2- Protection from natural enemies: Although groups may be more easily discovered by predators than individuals, individual group members may experience a lower risk of attack by predators because of factors such as increased vigilance and defence coming from other group members (Bertram, 1978; Lima, 1995). For instance, in meerkats, sentinels watch and alert their band when a predator is spotted, allowing all group members to rush to hide in their burrows. In social insects such as bees, colonies are organized and have guard members, who emit alert pheromones when a potential menace comes too close to the hive, triggering the formation of a group of bees that attacks the disturbing element (Bortolotti and Costa, 2014). Another benefit of group living is the dilution of risk of predator attack on any particular individual, when there is a limited number of prey that a predator can devour. This density-dependent process is purely probabilistic and does not require any complex or cooperative behaviour beyond aggregation (Bertram, 1978).

3- Protection from environmental conditions: Living in a group can shelter from climatic conditions. For instance, groups of emperor penguins save energy and are protected by social huddling from the cold during egg incubation. Insects are poikilothermic and individuals evaporate water through their respiratory aperture, which makes them prone to desiccation. The metabolic rate (i.e. energy expenditure) needed to maintain homeostasis decreases as group size increases, both by raising and stabilizing body temperature and by elevating relative humidity (Howe, 1962), which reduces desiccation risk for each group member (Tanaka, 2000).

4- Attacking and foraging together: Group foraging can provide benefits for nutrient acquisition, including being able to capture larger prey, to create aggregations of prey and to capture prey that are difficult or dangerous to catch. Group members make decisions that reflect a balance between efficiency and efficacy of obtaining food, defending their territory and protecting their young. For instance, after examining the context, the chances to catch the prey and the offspring's security, lionesses make the decision to attack a prey together and share this prey (Stander, 1992). Social insects also frequently use groups for searching for food. For instance, in red harvester ants, the foraging process is divided between three different types of workers (nest patrollers, trail patrollers, and foragers). While some individuals are guarding the anthill or taking care of the nest, others forage together in the wild and bring their loot back to the nest to share it with the rest of the anthill.

Costs

1- Competition for food, space, mates: When groups grow too large for a territory or limited resource, it can lead to competition among group members for space, food and even sexual partners, resulting in lower individual success. Large groups resulting in resource

over-exploitation may also lead to cannibalism (Hofer and East, 2008; Vijendravarma et al., 2013). Resource depletion can also adversely affect an individual's fitness when it reduces survival or reproduction. Insects frequently experience intraspecific competition for food due to their high fecundity and egg aggregation (Wertheim et al., 2001). At high densities, resource limitation can directly reduce fitness when it affects the probability of insect survival. Additionally, when it results in reduced body size of emergent adults (Grimaldi and Jaenike, 1984), it can affect other components of fitness such as lifespan (Tantawy and Vetukhiv, 1960), desiccation tolerance (Barker and Barker, 1980), male mating success (Ewing, 1964), and female fecundity (Atkinson, 1979; Tantawy and Vetukhiv, 1960).

2- Transmission of disease/parasites: Transmission of pathogens is expected to proceed more quickly in groups than through solitary individuals. Risks of parasite infection are particularly exacerbated in a group, because of the frequent and close contacts between individuals, and because of the close genetic relatedness often exhibited by group members, making them susceptible to the same parasites (Meunier, 2015; Otterstatter and Thomson, 2007; Schmid-Hempel, 1995; Wertheim et al., 2001).

3- Deteriorating environmental conditions: In large groups, overcrowding can lead to a deterioration of environmental conditions. For instance, eggs near the centre of egg batches frequently experience oxygen deprivation. This deterioration of the physical environment causes a delay in development and increases mortality (Imhof and Smith, 1979). In addition, increased humidity in hot spots of pest species boost fungal contamination of resources and carbon dioxide accumulates in the atmosphere, with potentially severe fitness consequences (Franco et al., 2021; Hardman, 1977).

4- Increased conspicuousness to natural enemies: Gregariousness can be disadvantageous when the group as a whole is more conspicuous to natural enemies than a solitary individual. For instance, wild horses aggregate into larger groups when biting flies are most active, although larger horse groups also attract more flies. However, the per capita attack rate is still lower in larger groups than small or intermediate groups (Sillen-Tullberg and Leimar, 1988). Moreover, natural predators may eavesdrop on the communication of their preys and use cues to locate them (e.g. chemical signature, echolocation) (Dicke and Sabelis, 1992; Jones, 2005; Simmons et al., 1979; Stowe et al., 1995). For instance, parasitoids exploit *Drosophila* aggregation pheromones to localise their hosts' breeding sites (Wertheim et al., 2003).

To conclude, individuals can benefit from joining groups when the group size is large enough to provide group advantages but small enough to avoid major costs caused by overcrowding. Individuals have to perpetually balance the potential costs and benefits in their decisions to join or avoid the group. What decision is optimal depends to a large extent on the environmental conditions, including light and temperature, the amount and quality of

food and other resources, and the density and prevalence of natural enemies, as well as on the decisions that other individuals make.

Allee effect, competition and dispersal

Aggregation not only affects individual performance, but can also influence population dynamic processes, including population growth, persistence and expansions. In this section, I will focus on few mechanisms that set lower and upper bounds of the size of local populations, including 1) Allee effect, 2) competition and 3) dispersal (Begon, 1996; Etienne et al., 2002).

Allee effect

Allee effects were first described in the 1930s (Allee, 1927). It refers to ‘a positive relationship between a component of individual fitness and either numbers or density of conspecifics’ (Stephens et al., 1999) (Figure 1). The Allee effect is also called inverse or positive density dependence, to highlight the contrast to the more generally considered negative density dependence that characterizes competition. This definition of Allee effects requires that measurable components of the fitness of an organism (e.g. survival probability or developmental rate of offspring) are higher in large groups than in a smaller one. Allee effects can arise from a variety of causes, as described above, and thus leads to a reduced per capita population growth rate at low densities (Allee, 1927; Courchamp et al., 1999; Dennis, 2002; Etienne et al., 2002; Kramer et al., 2009; McCarthy, 1997; Stephens et al., 1999). Inversely, negative density-dependence, due to overcrowding and competition, refers to a lower population growth rate at high local population densities (Courchamp et al., 1999) (Figure 1). Hence, Allee effects are dynamic phenomena that arise from the benefits of forming groups, and that manifest in several population processes, notably population growth or decline (Kramer et al., 2009).

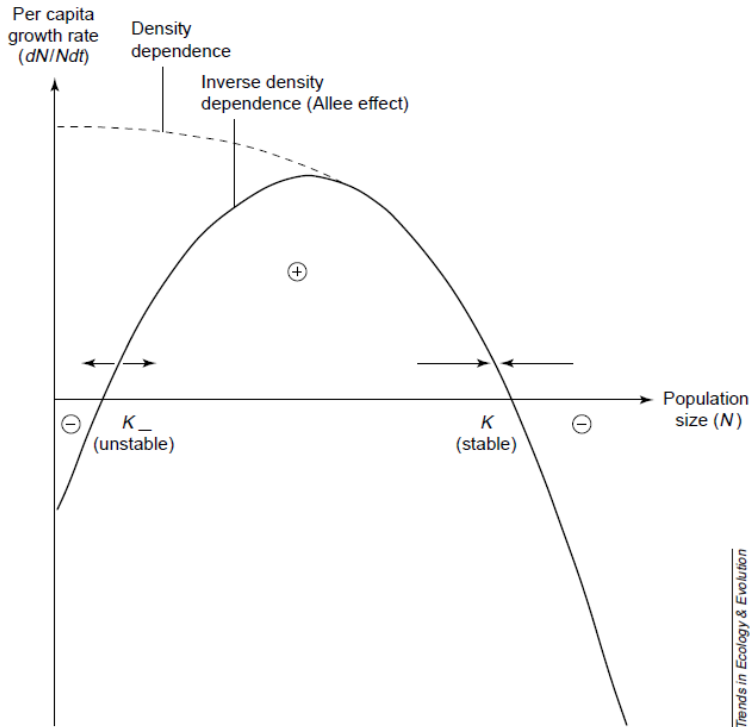


Figure 1: Positive and negative density-dependence curve (Courchamp et al., 1999).

Illustration of positive and negative density-dependence, from a simple mathematical model of population dynamics:

$$\frac{dN}{dt} = rN \left(1 - \frac{N}{K} \right) \left(\frac{N}{K_-} - 1 \right)$$

The per capita growth rate (dN/dt) is negative above the carrying capacity (K) and positive below K . However, in the presence of an Allee effect, it also decreases below a given population size, and can even become negative below a critical population threshold (K_-) which may lead to extinction.

Allee effects are considered to have two manifestations: component Allee effects and demographic Allee effects (Stephens et al., 1999). ‘Component Allee effects’ concern positive association between a component of fitness (e.g. viability, juvenile survivorship, fecundity, (Orr, 2009) and group size; while ‘demographic Allee effects’ occur when component Allee effects produce a positive association between per capita population growth and population size at low population density, and thus concern total fitness. Such a positive association may (but does not necessarily) give rise to a critical population size below which the population cannot persist (Stephens et al. 1999). Allee effects that cause critical population sizes are deemed strong, while Allee effects that do not result in critical sizes are deemed weak (Kramer et al., 2009).

The primary consequence of Allee effects is that it creates a critical threshold density below which the aggregation unit considered (e.g. population, colony, social group) cannot survive (Allee, 1927; Courchamp et al., 1999; Dennis, 1989; Ferdy and Molofsky, 2002;

Stephens et al., 1999). For example, this might correspond to the density below which it is so difficult to find a mate that reproduction does not compensate for mortality. Therefore, Allee effects may be crucial in conservation and management.

Competition

Apart from the positive density dependence, negative density dependence is also common within group and reflects mostly competition for limited resources both within and between species, as well as other costs associated with increasing group sizes (Ferdy and Molofsky, 2002). While individuals may benefit from joining a group, as their fitness in the group is higher than when solitary, at the same time, the benefits for the group decrease with additional group members due to increasing competition. As explained before, competition occurs naturally between living organisms that coexist in the same environment. According to Charles Darwin (Darwin, 1859), competition is the principal impetus in the evolution of life, as it is the driving force behind natural selection. In some species, such as meerkats, an adult female may kill offspring of other females so that her own offspring will benefit from reduced competition (Clutton-Brock et al., 1998). Aggression may also take place when individuals or groups defend their territory against other members of their species. For example, chimpanzee males patrol the boundaries of their territory and may be very aggressive toward males from outside their own troop, and they may attack and sometimes kill them (Wilson and Wrangham, 2003). Interspecific competition also happens between individuals of different species for the same limited resources. For instance, cheetahs and lions both feed on the same prey and may compete for these resources. In insects, aggressive behaviour toward conspecifics is also observed as in pavement ants that brutally attack other ants' colonies in their quest for new territory and resources, often resulting in thousands of dead ants (Hoover et al., 2016). Both intraspecific and interspecific competition are major forces in the adaptive evolution of species, favouring the individuals with traits and behaviours that do best in terms of survival and reproduction, while the less fit individuals decline in numbers and eventually becomes extinct.

Dispersal

The Allee effect and competition create a lower and an upper bound to group size. Coupled with individual dispersal, this can create an effective stable mean population density for the system as a whole (Etienne et al., 2002). For instance, the common breeding sites for fruit flies are ephemeral and patchily distributed. Without dispersal, a fruit fly population is doomed to go extinct, because the population number increases, leading to overcrowding, competition at some point in time and depletion of the ephemeral resources. With dispersal, the population can redistribute over the available resources, which may still lead to local overexploitation in some resources, but can be accompanied by more moderate and sustainable group sizes in other resources. Dispersal is thus very important because it is one of the mechanisms that couples the dynamics within and between populations and might help to overcome Allee effects and ensure long-term persistence of population (Etienne et al.,

2002). In addition, at the level of individual fitness, dispersal is a vital strategy that enables individuals to leave an unfavourable habitat, avoid predation or competition, find new ephemeral resources, search for mates, evade inbreeding, and colonize areas (Begon, 1996).

The evolution of sociality

Sociality is the degree to which a species tends to associate in social groups and form cooperative societies among conspecifics. Sociality is considered a species-specific trait – not an individual-specific trait. Diverse forms of sociality are found across insect taxa with more or less complex features such as communal nests, parental care, alloparental care, cooperative care and reproductive castes (Costa, 2018; Mas and Kölliker, 2008; Trumbo, 2012; Wong et al., 2013; Zabloutny, 2009). In insects, sociality ranges from solitary (e.g. cabbage white butterflies), to subsocial (e.g. burying beetles), to communal colonies (e.g. gregarious caterpillars such as oak processionary moths), to highly eusocial societies (e.g. ants, termites) (Costa, 2018; Leonhardt et al., 2016; Lin and Michener, 1972; Toth and Rehan, 2017; Wong et al., 2013; Zabloutny, 2009).

Sociality may seem at odds with Darwin's theory of evolution, as this theory is based on competition and survival of the fittest (Darwin, 1859). It does not easily account for altruistic behaviour. Yet, many species do engage in cooperative interactions, and such cooperation between individuals maintain animal societies due to their associated benefits for the individuals (Bergmüller et al., 2007; Lin and Michener, 1972). Evolutionary mechanisms of both conflict and cooperation pervade all of biology (Frank, 1998). Conflict and cooperation are not divergent phenomena, but are often intricately intertwined (Taborsky et al., 2021). For example, cooperation under the form of coalitions may be an efficient way to solve a conflict with third parties (e.g. lions, baboons) (Chakrabarti and Jhala, 2017; Packer, 1977).

Sociality largely centres around reproduction, as this is arguably the largest determinant of individual fitness. As such, the degree of social associations in a species is mainly shaped by the costs and benefits of reproductive strategies and behaviours. Females in particular are affected by the presence of others, both as competitors and collaborators, as their investments per offspring produced tend to be higher than those of males. Cooperation between females can be seen through alloparenting and helping behaviour that are central to female reproductive success. For instance, in many cooperative breeding species, reproduction is monopolised by the dominant pair within a group and subordinate females assist with offspring care (e.g. meerkat, African wild dogs, Ethiopian wolf) (Clutton-Brock et al., 2001; Coombes et al., 2018; Creel et al., 1997; van Kesteren et al., 2013). Competition can also be seen among conspecific females as females often compete for access to feeding territories or for raising their offspring (e.g. to give them access to resources, to establish their status within the group, to prevent them being evicted by other females) (Clutton-Brock,

2009, 1991; Clutton-Brock and Huchard, 2013; Kaufmann, 1983; Silk, 2007; Stockley and Bro-Jørgensen, 2011). Competition for reproduction among females is observed in a range of species, and is seen, for instance, through evictions from the group or frequent aggression towards other females (e.g. subordinates), which can lead to a down-regulation of their reproductive system, inhibit or delay sexual maturation, sexual receptivity and ovulation, or even cause spontaneous abortion or early offspring mortality (Clutton-Brock and Huchard, 2013; DeLong, 1978; Dunbar and Dunbar, 1977; Laws, 1929; Peyser et al., 1973; Rowell, 1970; Stockley and Bro-Jørgensen, 2011; Wasser and Barash, 1983, 1983; Wasser and Starling, 1988; Young, 2009). Reproductive competition thus limits cooperation between individuals.

To maintain large groups, mechanisms controlling internal conflicts and unifying group members into a cohesive and cooperative unit must exist. Mechanisms suppressing inter-individual competition are thus thought to be a major tenet of the evolution of sociality (Alexander, 1987; Frank, 2003; Frank, 2013). For instance, in eusocial insects, cooperation between individuals from the same nest is found through division of labour that occurs between reproductive and infertile females. While reproductive females mate and lay fertilized eggs, the majority of the female nest mates do not reproduce and instead forage, construct the nest, defend the territory and nurse the offspring of the reproductive ones (Heinze et al., 1994; Keller and Chapuisat, 2017; Kolmes, 1985; Robinson, 1992; Wilson, 1971). Sacrificing one's own reproduction for the benefit of another can be seen as a cooperation between individuals of the same nest and such cooperative behaviour can be evolutionarily favoured if the beneficiary is genetically related (Alexander, 1987; Bourke, 1999; Frank, 2003; Frank, 2013; Hamilton, 1964; Ratnieks and Reeve, 1992; van Zweden et al., 2012). This is called kin selection, and forms the foundation of the modern study of social behaviour. It states that a gene is favoured if it increases the inclusive fitness of individuals, comprising both its own reproduction but can also of its relatives (Hamilton, 1964, 1963; Kay et al., 2019; Maynard, 1964; Michod, 1982; Queller and Strassmann, 2002). Kin selection is thus part of natural selection and occurs when an animal engages in behaviour that benefits the genetic fitness of its relatives, even when this is at a cost to itself. This reproductive altruism and division of labour is key to eusociality.

Species with different levels of sociality modulate their reproduction depending on social context. Social context refers to the specific setting in which social interactions takes place. For instance, female rats exhibit ovarian synchrony with other female group members (McClintock, 1984; Schank and McClintock, 1997), fish adjust investment and egg size in offspring based on group size (Taborsky et al., 2007), ants and honeybees modulate oocyte size and reproduction with group composition (e.g. number of workers or larvae in the colony) (Mohammedi et al., 1998; Oldroyd et al., 2001; Traynor et al., 2014; Ulrich et al., 2016; Villalta et al., 2015), and gregarious cockroaches display advanced oocyte maturation and increased oviposition in the presence of conspecifics (Crall et al., 2016; Uzsák and Schal, 2012; Uzsák and Schal, 2013). The presence of conspecifics also modulates female

reproduction by stimulating egg production in *Rhagoletis pomonella* flies, an insect species that is considered solitary (Prokopy and Reynolds, 1998). Social modulation of reproduction thus appears to be a conserved phenomenon from mammals to insects and across a gradient of social complexity. However, despite this highly conserved phenomenon, the behavioural and physiological mechanisms of such social modulation of reproduction remain unclear and might be important for understanding the origins of sociality.

Sociability

Whereas sociality describes a species tendency to form social associations, sociability describes an individual's tendency to participate in group living and to engage in non-aggressive social interactions (e.g. feeding or roosting together, traveling in a group) (Bralten et al., 2021; Cote et al., 2008; Scott et al., 2018). In humans, for instance, highly sociable people report satisfaction from interacting with other people (e.g. they enjoy talking, the company of other people and physical closeness) and also tend to like being helpful and often act as 'helpers'. Some individuals prefer to be together with conspecifics all the time, while some other individuals prefer to be more solitary (Bralten et al., 2021). Such variation in sociability is found in various species. These variations can be maintained, because certain individual characteristics or personality traits match better or worse with a specific social environment, resulting in differences in costs and benefits for group living in different social groups (Cote et al., 2012; Cote and Clobert, 2007; Michelena et al., 2009).

Inter-individual differences in sociability occur naturally. This can be due to genetic factors, leading to variation in sociability traits, including with both highly sociable (e.g. Williams syndrome) (Deutsch et al., 2007; Jones et al., 2000) and unsociable phenotypes (e.g. autism, depression, bipolar disorder, schizophrenia, and anxiety) in humans (Chisholm et al., 2015; Dickerson, 2015; Green et al., 2015; Kupferberg et al., 2016; Lord et al., 2018; Rai et al., 2018; Reichman and Negron, 2001; Tigli Filizer et al., 2016; Winograd-Gurvich et al., 2006). Individual differences can also be the result of social environment, learning and exposure during development (Daniels and Plomin, 1985; Dopko et al., 2019; Kington et al., 2013).

Reduced social relationships and loneliness have important implications for health in multiple species and are among the top risk factors for human mortality (Holt-Lunstad et al., 2015, 2010). For instance, lowered sociability affects immune system functioning, sleep quality, mental health and increases cancer progression in rats, prairie voles and humans (Boen et al., 2018; Cohen et al., 1998; Grippo et al., 2008, 2007; Hermes et al., 2009; Kaushal et al., 2013; Kent et al., 2015; Leigh-Hunt et al., 2017; McNeal et al., 2014; Peuler et al., 2012; Pressman et al., 2005; Scotti et al., 2015). Social isolation also causes a disruption of the immune system in ants and zebrafishes, leading to a faster mortality (Forsatkar et al., 2017; Koto et al., 2015; Scharf et al., 2021). Even in *Drosophila*, considered as solitary insect, isolation and reduced social interactions have important negative impacts on health because

they lead to a faster tumour progression (Dawson et al., 2018) and a reduced lifespan (Ruan and Wu, 2008).

It is therefore well known that sociability is fundamental to life and health. However, little is known about the genetic and neural mechanisms that regulate sociability and explain its inter-individual variation.

***Drosophila melanogaster* as a model to study aggregation and sociability**

Drosophila melanogaster actively seek out the presence of conspecifics using aggregation pheromones, and form aggregations on breeding sites. In these aggregations, adult flies engage in mate finding and copulation, feeding and oviposition. This adult aggregative behaviour can be considered adaptive, due to an Allee effect that operates at the level of larval resource exploitation and survival (Wertheim et al., 2002b). The growth and survival of *Drosophila* larvae is positively correlated with group size at low densities. Hence, females that lay their eggs communally onto the same breeding site tend to have a higher reproductive success than solitary breeding females. Due to the communal egg laying in adult aggregations, larvae also tend to have aggregative distributions across potential breeding sites (Trienens et al., 2017; Wertheim et al., 2000, 2002b, 2006). However, larvae can also experience costs due to this aggregative female behaviour, when larval group sizes exceed the optimum or carrying capacity of the breeding site (Allee, 1927; Etienne et al., 2002; Stephens and Sutherland, 1999; Wertheim et al., 2002b). Then competition and even cannibalism starts to play a role (Narasimha et al., 2019; Vijendravarma et al., 2013). The risks and extent of these costs increase with the group size of the adult females and the number of eggs they lay on a breeding site. Consequently, the reproductive decisions that females make, both on engaging in communal egg laying and in the number of eggs they oviposit, are likely shaped by natural selection to optimize the costs and benefits of this behaviour. I thus predict that female reproductive behaviour is modulated by the presence of others and the group size. In this thesis, I aim to assess the mechanisms of this social modulation on female reproductive behaviour in *D. melanogaster*.

I use *Drosophila melanogaster* as model system, because its genetic tractability offers a unique opportunity to study the mechanisms of aggregative behaviour. Fruit flies are common insects in nature, they are easy to maintain in the lab because of their simple and inexpensive breeding and their short reproduction cycle. Powerful genetic tools have already been developed in this species, allowing an easy genetic manipulation of body structures, genes and receptors.

But is *D. melanogaster* a good system model to study social behaviour? Although *Drosophila* are traditionally classified as solitary insects, they show a variety of group behaviours including aggregation at food sources to feed, mate and lay eggs (Amrein, 2004; Bailly et al., 2021; Billeter and Levine, 2015; Duménil et al., 2016; Spieth, 1974; Wertheim et al., 2001, 2002b, 2002a). *Drosophila* adults actively form groups and attract conspecifics

to the same resources by using aggregation pheromones, which have been chemically identified and can easily be applied in experimental set-ups (Amrein, 2004; Bartelt et al., 1985; Billeter and Levine, 2015; Duménil et al., 2016; Wertheim et al., 2002a, 2005, 2006). To choose egg-laying substrates, fruit flies rely on social information gleaned from conspecifics (Battesti et al., 2012; Sarin and Dukas, 2009) and form social networks that modify their decisions (Pasquaretta et al., 2016; Schneider et al., 2012). Given a choice, females prefer to lay eggs with other females (Duménil et al., 2016), resulting in a higher offspring survival because larvae are more effective at combatting harmful fungi when in groups (Trienens et al., 2017; Wertheim et al., 2002b). Other benefits of group membership include improved memory (Chabaud et al., 2009; Muria et al., 2021), the opportunity to learn from one another (e.g. mate selection and mate copying) (Danchin et al., 2018; Kacsoh et al., 2015; Mery et al., 2009), a reduction of freezing responses in front of a danger (Ferreira and Moita, 2020) and an increase adult reproduction (Billeter et al., 2012; Krupp et al., 2008) and lifespan (Ruan and Wu, 2008). However, aggregating on breeding substrates also has costs as larvae compete for food, leading to cannibalism when resources are exhausted and dramatically reducing offspring survival (Allee, 1927; Courchamp et al., 1999; Etienne et al., 2002; Narasimha et al., 2019; Stephens and Sutherland, 1999; Vijendravarma et al., 2013; Wertheim et al., 2002b).

The costs and benefits of being in a group thus predict the existence of mechanisms that allow fruit fly females to adjust their behaviour and reproduction to the number of females and offspring already present on the resources, to maximize their own fitness. This therefore makes *D. melanogaster* a good model to study how social environment modulates individual behaviour and physiology. In **Chapter 2** of my thesis, I investigate how the presence of a group behaviourally and physiologically affects female reproduction, how the balance between cooperation and competition is shaped between group members and the mechanisms underlying the social modulation of oogenesis and egg-laying.

Similar to humans, fruit flies participate in social networks (Anderson et al., 2016; Saltz, 2011; Schneider et al., 2012; Simon et al., 2012). Social deprivation induces similar changes in metabolic gene expression in both species (Liu et al., 2018), increases aggression (Agrawal P. et al., 2020; Liu et al., 2011), shortens lifespan (Ruan and Wu, 2008) and even speeds up cancer progression (Boen et al., 2018; Dawson et al., 2018). Although humans and *D. melanogaster* are evolutionarily separated by approximately 600 million years (De Robertis, 2008), many biological, physiological, and neurological properties are conserved between the two species (Atkinson, 2011; Prüßing et al., 2013; Tolwinski, 2017; van Swinderen and Brembs, 2010), making *D. melanogaster* an unprecedented model to study the mechanisms of aggregative behaviour and sociability. In **Chapter 3** of my thesis, I explore sociability in *D. melanogaster*.

Reproduction in *D. melanogaster* and its social modulation

Reproduction is a fundamental feature of life, each individual organism existing as the result of reproduction. The presence of a group can behaviourally and physiologically affect female reproduction, including in fruit flies; it is thus essential to study which aspects of reproduction are modulated by the presence of others and what are the mechanisms underlying such social modulation of female reproduction. Hence, I will discuss in more detail several relevant aspects of female reproductive systems and behaviours in *D. melanogaster*.

Morphology of female reproductive system

Figure 2 presents the reproductive organ of *D. melanogaster* females. It occupies the posterior two-thirds of the abdomen and contains a pair of ovaries. Each ovary consists of 16-20 ovarioles, held together by a connective-tissue envelope. An ovariole represents a hollow structure and contains a series of growing egg chambers. Each ovariole opens into a lateral oviduct; the two lateral oviducts fuse into a common oviduct, which enlarges to form the uterus. The oviducts and uterus are innervated and have circular muscles to regulate egg movement (Bloch Qazi et al., 2003; Demerec, 1994). Eggs are fertilized by male sperm in the uterus. After copulation, sperm is immediately stored in paired spermathecae and a seminal receptacle. The receptacle accumulates approximately 80-90% of the stored sperm (Fowler et al., 1968). Three regions can be recognized in each ovariole of the mature ovary: a terminal filament (at the most anterior tip of the ovariole), a germarium (the most anterior region of the ovariole), and the vitellarium.

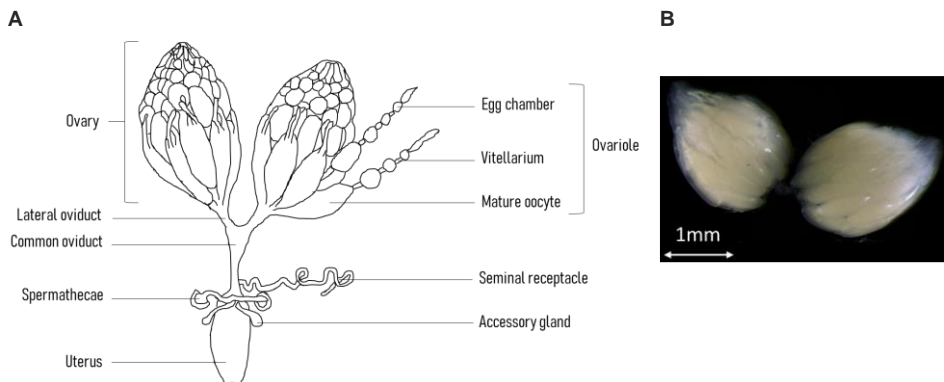


Figure 2: Overview of a mature *D. melanogaster* female reproductive system. (A) Schematic representation of the fruit fly female reproductive system (inspired by Bloch Qazi et al., 2003 and Ogienko et al., 2007). (B) Photo of ovaries from a mated female *D. melanogaster* (Photo credits: Tiphaine Bailly).

Oogenesis, ovulation and fertilization

Oogenesis

Oogenesis in *Drosophila* is asynchronous and eggs are produced continuously (King, 1970). Each ovariole of the ovaries acts as an egg production line. Egg chambers arise from the germarium situated at the anterior of the oocyte and progress through 14 morphologically distinct stages (Figure 3) (Bastock and Johnston, 2008; Soller et al., 1999; Spracklen and Tootle, 2013). Each egg-chamber is composed of an oocyte, nurse cells that together form the germ cell cyst, and are surrounded by the follicle cells. Follicle cells synthesize some of the yolk protein that will be deposited into the oocyte – a process called vitellogenesis – as well as the proteins of the vitelline envelope and chorion that cover and protect the oocytes (Bloch Qazi et al., 2003; Brennan et al., 1982; Isaac and Bownes, 1982). At the end of these stages, the oocytes are mature (stage 14) and can be stored in an arrested and inactivated form in the ovaries. Oocytes arrest in metaphase of meiosis I.

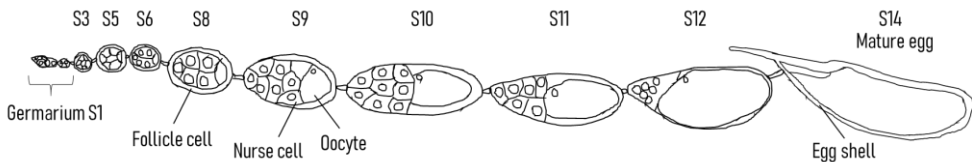


Figure 3: Overview of *Drosophila* oogenesis. Different stages of oogenesis are presented here, stage 14 being the most advanced stage which corresponds to fully developed eggs that are mature and ready for ovulation.

Ovulation

Ovulation is the delivery of a mature oocyte from the ovary into the uterus. In this process, the oocyte is released from its follicle, squeezed out of the ovary, and pushed through a lateral oviduct into the common oviduct, coming to rest in the uterus. Ovulation triggers the process of oocyte activation, causing the transition from an oocyte into an egg (ovum). Indeed, ovulation makes an oocyte available for fertilization, regulates the rate of oocyte release, and initiates changes in the oocyte (e.g. decrease of egg envelope's permeability, increase of vitelline membrane protein cross linking, resumption of meiosis and initiation of translation) that prepare it for fertilization (Bloch Qazi et al., 2003). Mating increases female ovulation rate and females produce a high number of mature oocytes (Heifetz et al., 2001). Ovulation is under neuronal control because octopaminergic neurons innervate female reproductive tract, allowing the contraction of oviducts that releases the mature oocytes (Bloch Qazi et al., 2003; Li et al., 2015; Rodríguez-Valentín et al., 2006).

Fertilization

Fertilization is the union of male and female gametes to form a new individual. It takes place after the ovulation, when the female egg is in the uterus. In the uterus, the egg is fertilized by male sperm coming from the female sperm storage organs (i.e. the seminal

receptacle and the paired spermathecae) (Bloch Qazi et al., 2003; Demerec, 1994; Hoffmann, 1995; Lefevre and Jonsson, 1962; Tram and Wolfner, 1999). Sperm storage allows sperm from a given mating to be used long after the male and female have separated (up until about 2 weeks). *D. melanogaster* females are polyandrous and, due to the sperm storage organs, a female can also retain sperm from more than one male within her reproductive tract; this process is called the female sperm precedence (Birkhead and Hunter, 1990; Bloch Qazi et al., 2003; Chapman et al., 1995; Lefevre and Jonsson, 1962; Schnakenberg et al., 2012; Zeh et al., 1997). This provides a certain fertility insurance, provides different genotypic choices for the progeny and reduces the risk of genetic incompatibility. Female remating is very common and frequent in *D. melanogaster*, and females often carry sperm of 4 to 6 males in their sperm storage organs (Imhof et al., 1998; Ochando et al., 1996). Therefore, sperm competition is high and males need to compete for access to fertilizations (Birkhead and Moller, 1998; Birkhead and Hunter, 1990). Sperm competition and sperm preference are two important processes in fertilization.

Oviposition

Oviposition, also called egg-laying, is the deposition of eggs by the female onto a substrate. Although *D. melanogaster* mature virgin females are able to spontaneously ovulate at a low rate, only fertilized eggs are viable. Figure 4 presents a *Drosophila* egg. Its opaque, thick and tough membrane is called the chorion. The two respiratory filaments and the micropyle are extensions of this chorion. The respiratory filaments are the major region of gas exchange. The chorion is a structure that maximizes gas exchange and minimizes water loss. The micropyle is a tiny channel that leads to the ovum, and it is through this channel that sperm penetrates, as the egg passes through the uterus. (Demerec, 1994; Gilbert and Barresi, 2016).

After the deposition of the egg on a substrate, the new individual develops in several stages (i.e. embryonic stage, larva stage with 1st, 2nd and 3rd instars, pre-pupa and pupa stage) until the birth of a new adult fly (Gilbert and Barresi, 2016).

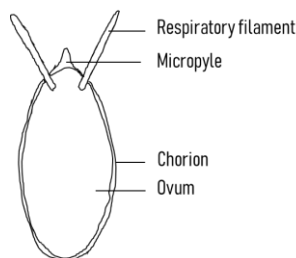


Figure 4: Schematic representation of a *Drosophila* egg on dorsal view. The egg of *D. melanogaster* is about 0.5 millimeters long. The unfertilized female gamete (ovum) is covered by a membrane (chorion) that provides protection from desiccation. Fertilization with sperm occurs through the micropyle, which is located between two membranous extensions of the chorion that allow for gas exchange (respiratory filaments) during embryonic development.

Oviposition site selection

Choosing a suitable oviposition site is a key decision for egg-laying animals such as fruit flies which lay their eggs on appropriate food substrate and do not provide additional parental care. The survival, and even the fitness of the future generation, are dependent on this substrate decision. This is particularly important for animals where the offspring is confined to these oviposition sites for their development. This is the case for larvae of *D. melanogaster*, which breed on ephemeral resources (i.e. decomposing and rotting fruit items), and can explore only a restricted area in search of alternative feeding sites. Unfavourable oviposition sites may result in mortality or hamper growth (Grimaldi and Jaenike, 1984; Vijendravarma et al., 2012). The latter could result in smaller adult body size, which negatively affects fecundity, mating success, starvation resistance and health (Edmunds et al., 2021; Fricke et al., 2008; Klepsatel et al., 2020). Location of a suitable oviposition site is therefore of great importance.

Female flies can explore their environment before selecting an oviposition site. Visual, olfactory and gustatory cues are involved in the substrate choice, both at long range and after arrival on the substrate. Before laying eggs, female flies evaluate the composition of the resource through olfactory receptors in the antenna (Duménil et al., 2016; Dweck et al., 2013) and gustatory receptors present in their proboscis and ovipositor (Gorter et al., 2016; Joseph et al., 2009; Yang et al., 2008). *Drosophila* females can also visually detect the presence of predators (e.g. parasitoid wasps) before laying eggs on a substrate (Ebrahim et al., 2021; Sadanandappa et al., 2021).

In *D. melanogaster*, female oviposition decisions can be influenced by a complex mix of social, nutritional, environmental and genetic factors. These factors can be: social composition of the resident group on a breeding site (Del Solar and Palomino, 1966), larval conditioning of the medium (Dawood and Strickberger, 1968), egg and larval density on the breeding site (Lewontin, 1955), competitive interactions with conspecifics (Barker and Podger, 1970; Bentley and Day, 1989; Soliman, 1971), presence of predators (Kacsoh et al., 2015; Sadanandappa et al., 2021), exposure to temperature, food quality and abundance (Gorter et al., 2016; Herrero, 2012; Mainardi, 1968; Richmond and Gerking, 1979; Soliman, 1971), surface texture or colour of the substrate (Carfagna and Lancieri, 1971; Chess and Ringo, 1985; Marcus et al., 2018; Rockwell and Grossfield, 1978; Solar et al., 1974; Volpe et al., 1967), prior experience and exposure to particular food or compounds (Abed-Vieillard and Cortot, 2016; Flaven-Pouchon et al., 2014), social learning about egg-laying substrates (Sarin and Dukas, 2009). Fruit fly females moreover avoid a substrate when competitor species (e.g. parasitoid wasps) already use it (Ebrahim et al., 2015; Lefèvre et al., 2012; Sadanandappa et al., 2021).

The quantity and quality of food are main factors for selecting a substrate, because both directly determine the offspring's chance of survival. Fermentation products of yeast (e.g. acetic acid, acetone, 2-methyl-1-butanol, 2-phenylethanol, ethanol) growing on the fruit attract flies for laying their eggs (Becher et al., 2012, 2010; Duménil et al., 2016; Eisses,

1997; Gou et al., 2014; Richmond and Gerking, 1979; Zhu et al., 2003). While virgin females are attracted by food containing sugar over food containing yeast (Ribeiro and Dickson, 2010), mated females seek out yeast, whose nutrient-rich content is essential to their egg production and for their offspring's development (Becher et al., 2012; Carvalho et al., 2010; Eisses, 1997; Gou et al., 2014; Joseph et al., 2009; Terashima and Bownes, 2004). Mating induces a behavioural change or a preference switch for substrate odours that are suitable for egg-laying. For instance, *D. melanogaster* females have an innate positional aversion to acetic acid but when they need to lay eggs, they are attracted by acetic acid substrate (Joseph et al., 2009). Acetic acid attraction is activated each time an egg is being squeezed through the reproductive tract as it has been found that the presence of egg(s) in the internal reproductive tract is sufficient to trigger acetic acid attraction regardless of egg-laying (Gou et al., 2014).

Social environment modulates female reproduction

Signals left by conspecifics on a substrate

Chemical cues left behind by sender flies act as a public source of information for responder flies about egg-laying sites which can affect their behaviour (Duménil et al., 2016; Lin et al., 2015; Symonds and Wertheim, 2005; Wertheim et al., 2002a). These egg-laying sites are utilized for social exchange of information, from which both senders and responders may benefit. Naïve females learn about egg-laying sites through cues left by mated females. Indeed, *D. melanogaster* mated females can influence the choice of egg-laying site of other females through pheromonal marking, i.e. combination of cVA (a male volatile chemical compound transferred to female during copulation) and sex -and species- specific cuticular hydrocarbons produced by both mates (Duménil et al., 2016; Tinette et al., 2004). The quality of substrates is also essential when choosing a communal oviposition site. Both males and females are attracted more towards odours from males and mated females when these are combined with fresh yeast or even acetone and ethyl acetate (yeast-fermentation products), (Bartelt et al., 1985; Duménil et al., 2016; Lebreton et al., 2012). The egg-laying patch decision by responder females is therefore a balance between food patch quality and the transfer of social information from sender females that can benefit both senders and responders by leading to their offspring cooperation and increasing their survival.

Social context modulates female reproduction

Female reproductive decisions can be modulated by their social environment in various distinct ways. For instance, *D. melanogaster* females remate more often as the number or genetic diversity of males increases in the group (Billeter et al., 2012; Gorter et al., 2016; Krupp et al., 2008). Females also eject sperm after mating faster when they perceive the presence of other females (Billeter et al., 2012; Laturney and Billeter, 2016). In addition, mated females aggregate and attract other females to an egg-laying substrate, leading to communal egg-laying (see 'Signals left by conspecifics on a substrate' section). This shows

a social attraction for egg-laying. Once on a food patch and in a group, fruit flies may further modulate their behaviour depending on group density. For instance, females can affect or regulate group size through aggression (Bath et al., 2017). This behaviour is qualitatively and quantitatively different from male-male aggressions which often occur during courtship and mating, while females rather show aggressive behaviours toward one another when placed on a food source containing live yeast. A female may lunge at another female, erect her wings or push another by extending her legs, head-butting, and fencing (Ueda and Kidokoro, 2002). Mating may increase aggressivity in females *D. melanogaster* and sperm or other ejaculate components (more precisely, sex peptide) released from male into the female stimulates this aggression between females (Bath et al., 2017), competing for egg-laying substrates.

Components and hormones that modulate female reproduction

Hormones, proteins or peptides from both males and females can control different aspects of the *D. melanogaster* female reproductive process (e.g. vitellogenesis, oogenesis, ovulation, egg-laying). I will focus on and describe here only two components that are relevant to this thesis.

Juvenile hormone

Juvenile hormone (JH) is the major gonadotropin in female insects, including *D. melanogaster*. JH is synthesized by the *corpora allata* (CA) (Noriega, 2014; Shinoda, 2016), which is positioned just posterior of the brain. The regulation of JH synthesis is influenced by developmental, environmental and physiological cues, such as nutrition (Schal et al., 1993; Shinoda, 2016), temperature (Kurogi et al., 2021) and mating (Bownes and Rembold, 1987; Moshitzky et al., 1996; Sliter et al., 1987). JH regulates multiple physiological events, including metamorphosis and the development of immature insects (Noriega, 2014; Riddiford, 2012; Smykal et al., 2014), but also vitellogenesis and oogenesis in adult insects. Indeed, JH regulates oocyte maturation and the synthesis of vitellogenin (i.e. a protein synthesized by female insects and used to produce yolk in the fat body). This functions as a control point of vitellogenic oocyte development around stage 9 (Bilen et al., 2013; Dubrovsky et al., 2002; Kelly et al., 1987; Meiselman et al., 2018; Noriega, 2014; Riddiford, 2012; Santos et al., 2019; Soller et al., 1999; Wilson, 1982). At this stage, JH stimulates oogenesis (oocyte development, oocyte maturation, egg chamber resorption) by inducing uptake and synthesis of yolk proteins by the developing oocytes (progression of vitellogenic oocytes) (Berger and Dubrovsky, 2005; Bownes and Hames, 1978; Jindra et al., 2013; King, 1970; Riddiford, 2012; Sheng et al., 2011; Soller et al., 1999, 1997; Wu et al., 2021).

Social environment can modulate JH production. In German cockroaches, social interactions stimulate JH production which leads to faster oocyte development and to an acceleration of female oogenesis when in a group (Uzsák and Schal, 2012). Stressful environmental factors such as starvation or thermal stress can also indirectly modulate JH production by the increase of ecdysone titers which lead to an inhibition of JH production

and arrest oogenesis (Meiselman et al., 2017, 2018; Soller et al., 1999; Terashima et al., 2005; Terashima and Bownes, 2004).

Ecdysone

Ecdysone is a steroidal prohormone of the major insect molting hormone 20-hydroxyecdysone (20E), which is synthesized in the prothoracic glands in the insect prothorax (Hoffmann, 1980; Riddiford et al., 2000). It is secreted to hemolymph, and oxidized to 20E in peripheral tissues such as the fat body (Riddiford et al., 2000). Its production is under the control of a neurohormone of the brain, the prothoracicotropic hormone (Hoffmann, 1980; Riddiford et al., 2000).

Ecdysone triggers molting of the larval instars, metamorphosis during the pupal stage (Garen et al., 1977; Hoffmann, 1980; Riddiford et al., 2000), and plays a role in the adults' vitellogenesis process. It is also required for oogenesis synchronization and the regulation of oocyte maturation (Bownes, 1989; Buszczak, 1999; Garen et al., 1977; Hoffmann, 1980; Swevers and Iatrou, 2009). Ecdysone plays both positive and negative roles in the regulation of oogenesis in *Drosophila* (Soller et al., 1999; Swevers and Iatrou, 2009). Ecdysone stimulates yolk protein synthesis by the fat body (i.e. high rate of vitellogenin synthesis) as well as yolk protein uptake from the ovary immediately after eclosion when follicles are at the previtellogenic stages (Carney and Bender, 2000; Jowett and Postlethwait, 1980; Postlethwait and Shirk, 1981; Richard et al., 1998; Swevers and Iatrou, 2009; Wu et al., 2021). Ecdysone has also negative effects on ovarian follicle development, at stages 8 and 9, which correspond to the initiation of vitellogenesis (Drummond-Barbosa and Spradling, 2001; Swevers and Iatrou, 2009): in adverse physiological conditions that do not support oocyte maturation (e.g. starvation, stress, absence of mating), ecdysone titers rise and cause nurse cell apoptosis and follicle degeneration in stages 8 and 9, and block vitellogenesis of eggs (Bownes, 1989, 1986; Buszczak, 1999; Garen et al., 1977; Hoffmann, 1980; Soller et al., 1999; Swevers and Iatrou, 2009; Walker et al., 1987).

Ecdysone and juvenile hormone can interactively govern oogenesis in response to physiological state and environmental conditions. In beneficial physiological conditions, the effects of ecdysone are counterbalanced by increased production of JH which stimulates initiation of vitellogenesis (Meiselman et al., 2017; Soller et al., 1999). For example, in the case of mating, the progression of follicle development is mediated by the transfer of the sex peptide to the female (Chen et al., 1988; Moshitzky et al., 1996), that acts on the *corpora allata* to stimulate the production of JH (Moshitzky et al., 1996), which counteracts the apoptotic effect of ecdysone on vitellogenic follicles (Soller et al., 1999). A correct balance between JH and ecdysone is thus essential for oogenesis synchronization and for the progression of vitellogenic follicle development in *Drosophila* (Soller et al., 1999; Terashima et al., 2005).

Thesis overview

In this thesis, I investigate how social environment modulates *D. melanogaster* female reproduction. I focus on both the function of the social modulation of oogenesis and egg-laying behaviour and the mechanisms that are involved in this phenomenon (**Chapter 2**). More precisely, the trade-off between cooperation and competition of laying eggs in group is discussed in **Chapter 2**. The effect of light on oogenesis and egg-laying and its interaction with social environment is also explored in **Chapter 2**. To understand the mechanisms that regulate an individual's response to these environmental stimuli, I moreover investigate the hormonal pathways involved in such modulation of female reproduction (**Chapter 2**). Furthermore, how females detect the presence of a group is determined (**Chapter 2**) by the use of genetic mutants for sensory modalities and by silencing sensory neurons with the Gal4-UAS system (explained in **Box 1**).

Then, to test whether this social facilitation of egg-laying is a widespread phenomenon among wild-type *D. melanogaster* lab strains, I further study the egg-laying behaviour of female from different wild-type strains in response to a group (**Box 2**). In the **Chapter 3** of my thesis, I additionally examine the variation in the response to a group among *Drosophila* Genetic Reference Panel (DGRP) flies, a resource of wild-type, inbred and sequenced *D. melanogaster* lines. For that, I explore the variation in sociability between these lines through a multidimensional approach, by quantifying sociability through three behavioural assays that each captures different features of social interactions (**Chapter 3**). In the last Chapter, I give a general synthesis of my findings and offer perspectives for future research (**Chapter 4**).

Box 1

Gal4-UAS binary system

Tiphaine P. M. Bailly

The Gal4-UAS binary system is a powerful technique commonly used in *Drosophila melanogaster* for studying the expression of genes and for targeted manipulation (Brand and Perrimon, 1993). Figure 1 presents a schematic overview of the Gal4-UAS binary system. The Gal4 gene encodes the yeast (*Saccaromyces cerevisiae*) transcription activator protein Gal4. Gal4 is controlled by a *D. melanogaster* promoter, or driver gene, to ensure that Gal4 is only expressed in tissue in which this driver is activated. One of the main targets of Gal4 is a cis-regulatory site (DNA sequence) called Upstream Activating Sequence (UAS). UAS is an enhancer to which Gal4 specifically binds to activate gene transcription. Gal4 is thus expressed in specific tissues by a nearby enhancer, and Gal4 protein binds to engineered UAS sequences to drive the expression of a specific gene in those tissues. To manipulate or visualize target tissue, the appropriate Gal4 line (F0) is crossed to the UAS line (F0) that serves the manipulation's purpose. The offspring of this cross (F1) has both transgenes in its genome and the UAS transgene is expressed in the tissue of interest as shown in Figure 1.

In **Chapter 2**, the involvement of specific sensory modalities and neurons are questioned for their role in group detection in the context of egg-laying advancement. I thus use the Gal4-UAS system to manipulate and silence specific neurons. Female offspring harbouring both transgenes are tested for group detection and egg-laying timing.

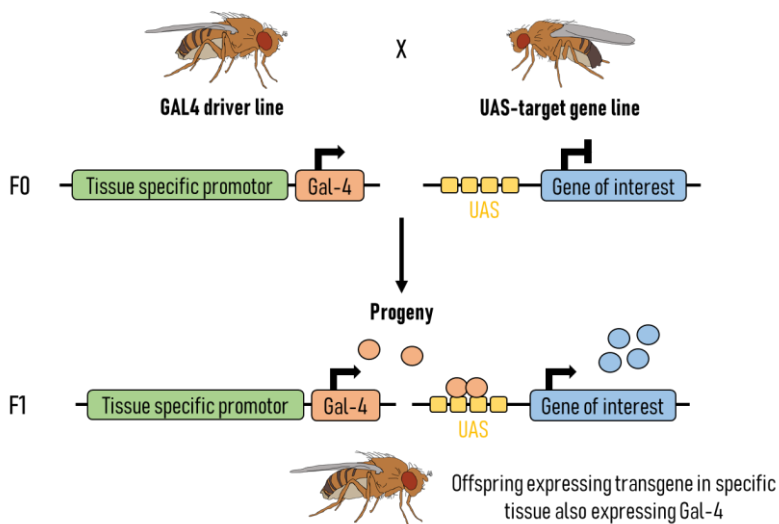


Figure 1: Gal4-UAS binary system. A schematic overview of the Gal4-UAS system which drives expression of a gene of interest in a specific tissue. Tissue-specific promoters drive expression of GAL4 that results in transcriptional activation from the GAL4 binding sites (UAS) to drive expression of a gene of interest. This system is widely used for expression of fluorescently tagged proteins, for gene knockdown (by RNAi) and for gene silencing (by Kir2.1). A tissue-specific driver GAL4 line (F0) can be crossed to UAS transgene complexes line (F0) to generate progeny (F1) with the transgene of interest expressed in the target tissue. The F1 offspring are then tested for specific questions.

