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Transgenerational Epigenetic Inheritance of Learning and Memory: Can Flies Tell Us The Truth?*

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Abstract Learning and memory is a delicate process of acquiring, storing and reconsolidating the knowledge with a behavioral output, which is indispensable for animals to adapt to their living environment. Defects in learning and memory contribute to some psychiatric disorders such as schizophrenia, depression and Alzheimer's disease, and are recently reported to be inheritable from the parental generation to their offspring. However, it is not clear currently what the mechanism is underlying the learning and memory inheritance due to the lack of animal models. In this perspective type of mini-review, we first briefly summarize the current understanding of the molecular basis, neural circuit and transgenerational inheritance of learning and memory. We then focus on discussing the possibility of using *Drosophila* as an animal model to study the transgenerational inheritance of learning and memory and propose potential strategies to achieve the goal.

Key words transgenerational inheritance, learning and memory, acquired behavior, epigenetic marks, *Drosophila*
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Learning and memory are generally defined as a process that requires an environmental input and a responsive behavioral output. Learning is a behavioral process of acquiring knowledge from transient inputs, and memory is a physiological process of storing and reconsolidating the knowledge that an animal previously learned. Higher organisms are capable of learning from experiences and adapting themselves to the dynamic environment with their memories that help them to discriminate potential beneficial and harmful matters. Learning and memory are also indispensable in all aspects of humans, determining the way and quality of our lives according to what we remember and forget. Defects in learning and memory contribute to psychiatric disorders such as schizophrenia, depression and Alzheimer's disease, and affect the cognitive capability and life quality of such patients and their descendants [1–3]. In the past decades, the molecular and neural circuit mechanisms underlying the acquisition, storage and reconsolidation of memory have been largely uncovered and summarized in many comprehensive reviews [4–6], establishing an elementary conceptual framework of learning and memory. In this mini-review, we give a brief summary of the molecular mechanisms of learning and memory, and focus on examining the discoveries on the transgenerational epigenetic inheritance of acquired behaviors underlying the appetitive and aversive memory. In the end, we

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highlight that *Drosophila* is a potentially excellent animal model to study the transgenerational epigenetic inheritance of learning and memory, and possible strategies for further investigations are proposed.

1 Molecules and neural circuits underlying the memory storage and reconsolidation

Memory refers to processing of the learnt information, and displays many common properties with synaptic plasticity, suggesting that synaptic plasticity is involved in memory storage and the strength of synaptic plasticity is linked to memory reconsolidation [7–8]. Studies of the mammalian behavioral conditioning have revealed that memory is mainly stored in the hippocampus and retrieved in the medial temporal lobe of the brain [5]. In flies with classical conditioning of behavioral paradigms, the mushroom body (MB) of the central brain is found to be required for the olfactory memory[9–10], whereas the fan-shaped body (FB) is important for the visual pattern memory[11–12]. It is thus incontrovertible that the genes regulating the synaptic structure and plasticity of these brain regions are crucial for the memory formation[4–5]. However, given that the timescale of the gene transcription and protein turnover is several hours, it is questionable that the long-term memory last for even a life time long if the memory is only stored in proteins. It is then becoming evident that the perpetuating modifications but not the molecules themselves would be the key features of molecular changes [13], raising the proposition that epigenetic mechanisms have the possibilities to preserve memories over the entire life time [6]. In the following parts we will briefly summarize the discoveries about the storage, reconsolidation and epigenetic regulation of memory (Figure 1a).

1.1 Short-term memory storage

Earlier studies in invertebrates with simple models such as gill-withdrawal reflex in *Aplysia*, flight trace in honeybees and olfactory learning in fruit flies started the era of memory[14–17]. The short-term memory that lasts for from several minutes to several hours was first reported in the *Aplysia* studies on gill-withdrawal reflex[18]. The sensory and motor neurons of *Aplysia* are both found to be required for gill-withdrawal reflex. Further serotonin and its downstream effector cAMP (cyclic adenosine monophosphate) were reported to regulate the release of neural transmitter glutamine and to control the synaptic connections between the sensory and motor neurons[18]. Later studies, using the classical or operant conditioning, show that gill-withdrawal reflex is increased notably when conditioned stimuli are applied [19–21]. After the conditioned stimulus, the cAMP signal is activated through increased influx of calcium ions, strengthening the synaptic connections between sensory and motor neurons[19].

1.2 Long-term memory reconsolidation

Earlier studies in different invertebrate models show that the duration of memory storage occasionally lasts for days, weeks, or even a longer time, which is referred as the long-term memory [5]. The synaptic connections in the long-term memory are further enhanced, comparing to that in the short-term memory, which require regulator PKA (the cAMP-dependent protein kinase) and its effector MAPK (mitogen-activated protein kinase) [22–23]. The PKA and MAPK signals activate the target gene expression through preventing the binding affinity of CREB-1 (cAMP response element binding protein-1) to DNA [24]. Further studies reveal that CREB-1 activates the transcriptional factor C/EBP (CCAAT-enhancer binding protein) and initiates the downstream gene expressions, which is crucial for the synaptic growth and connections [25–28]. Unlike the positive role of CREB-1 in memory reconsolidation, CREB-2 suppresses the synaptic growth and inhibits the long-term memory reconsolidation[29]. It is a conserved pathway for memory reconsolidation that CREB-1/2 controls the expression of target genes important for the long-term synaptic connections[30].

1.3 Epigenetic regulation of the formation and maintenance of memory

Epigenetic mechanisms, including DNA methylation, histone modifications and noncoding RNAs, have been shown to be involved in memory formation and maintenance [6–7], also see Figure 1a. Earlier studies reported that an inhibitor targeting DNA methyltransferase (DNMT) affects the formation of fear memory, implying that the changes of DNA methylation are involved in memory formation [31–33]. Recent studies showed that mice lacking a DNA demethylase Tet 1 (ten-eleven translocation methy-cytosine dioxygenase 1) display impaired fear memory and spatial memory, further suggesting that Tet 1 is important for memory formation[34–35]. The transcription of genes that are crucial for synaptic growth are changed in the Tet 1 knockout mice, suggesting that

transcriptional events mediated by DNA methylation regulate the memory formation [36]. However, it is still unknown how the environmental inputs can change the status of neural DNA methylation.

Histones can be modified by various chemical groups such as methyl-, acetyl-, phospho-, and sumo-groups, resulting in changes of gene transcription [6]. The first report linking histone modifications to memory formation was from the observation that acetylation level of histone H3 in the hippocampus is increased after contextual fear conditioning [37]. Following these findings, many labs tested possible roles of the histone acetyltransferases (HATs) and histone deacetylases (HDACs) in different behavioral paradigms and reported that histone acetylation is crucial for auditory, spatial and contextual memory formation and reconsolidation [38–43]. It has also been reported that histone phosphorylation and methylation are important for memory formation as well [44–47]. The H3K9me2 (a mark for transcriptional repression) and H3K9me3 (a mark for transcriptional activation) show opposite effects on memory formation [48], both of which are the epigenetic marks on the promoter region of BDNF (brain-derived neurotrophic factor) gene [46].

Recent studies of noncoding RNAs have shown that the microRNA (miRNA) plays important roles in the memory formation in the fear conditioning tasks [49–51]. The expression level of miRNA targets increases (e.g. miR-34a) or decreases (e.g. miR-182) after fear conditioning, and consequently miR-34a promotes, whereas miR-182 inhibits, the fear memory reconsolidation [51]. Further studies revealed that miR-34a promotes fear memory reconsolidation through the Notch signaling pathway, whereas miR-182 regulates the transcription of actin network-related genes to prevent the memory reconsolidation [51].

In summary, these epigenetic mechanisms underlying the memory formation and maintenance are at the level of transcriptional regulation of various target genes that are crucial for synaptic growth and connections. More efforts will be needed to investigate how the epigenetic mechanisms link the environmental stimuli to acquired memory formation, and whether the inheritance of epigenetic marks is responsible for the transgenerational inheritance of acquired behaviors.

2 Transgenerational epigenetic inheritance of acquired behaviors through learning and memory

The molecular and circuit bases of learning and memory are becoming more and more explicit, and are shedding light on the pathologies and treatments of some of the psychiatric diseases [1–2]. However, as the psychiatric diseases have been reported to have strong heritable risks [3, 52–53], it is essential to understand the heritable mechanisms of acquired behavioral traits. The acquired physiological traits (non-behavioral traits) have been recently reported to be transmitted from the parents to the descendants through an epigenetic mechanism in the germline [54–55], whereas the acquired behavioral traits can be inherited to the descendant generations through not only the way of epigenetic transmission but also the way of social transmission [56]. Social transmission requires the interaction between the parent and the offspring and does not involve the changes of genetic or epigenetic information; whereas the epigenetic transmission requires the epigenetic alterations in germline without any interaction between the parent and the offspring [1, 56]. In the following sections, we summarize the recent studies on the transgenerational inheritance of acquired behaviors and discuss the possibilities of germline mediated transgenerational inheritance (also referred as transgenerational epigenetic inheritance) of behavioral traits (Figure 1b).

2.1 Transgenerational inheritance of behavioral traits in different memory conditioning

The first study on transgenerational inheritance of learning and memory emerged as that the learning ability of the rats can be detected in their offspring [57]. A following study showed that the parental rats with a high frequency of licking and grooming behavior in the Morris water maze produce the offspring with similar high frequency of licking and grooming behavior, indicating that the high cognitive behavior acquired in the parental generation (F_0) can be transmitted to the descendant generation [2].

Recent mechanistic studies on the transmission of behavioral traits have given us a clear definition of transgenerational inheritance and its difference from intergenerational inheritance [58–60] (Figure 1b). Both of these two routes of inheritance require specific epigenetic marks in the germ cells; and the essential difference between them is whether the germ cells are

directly exposed to the environmental stimuli that cause the epigenetic modifications. The intergenerational inheritance involves the epigenetic modifications in the germ cells caused by the environmental stimuli, whereas the transgenerational inheritance involves epigenetic modifications from the parental generation but not direct effects of the environmental stimuli (Figure 1b). For example, when the germ cells of the parent (F_0) or the germ cells of unborn offspring (F_1) have already experienced directly the environmental exposures, the behavioral traits caused by these environmental exposures can be transmitted from the parent (F_0) to the descendant generations (F_1 and F_2). In this case, the transmission of acquired traits lasting for two generations belongs to intergenerational inheritance, and the transmission lasting for more than two generations (such as F_3) belongs to transgenerational inheritance.

In this context, one of the striking examples has been recently reported in the vinclozilin-exposed rats[61]. The parental rats displayed anxiety-like behavior when exposed to vinclozilin, an anti-androgen fungicide. Notably, this anxiety-like behavior was also detected in the F_1 offspring and lasted until the F_3 generation[61]. It is also interesting that the acquired anxiety-like behavior in the descendants affected the mate preference for the non-exposed rats. Further investigations showed that the transcriptional profiles were remarkably changed in the brain of F_3 generation rats [61]. Other examples in chickens and mice also demonstrate that the learned behaviors and long-term memory can be transmitted to the descendant generations when their parent(s) experience the enriched environment such as various toy objects for playing, bigger population of animals for complex social behaviors, various physical activities or repeated training [52, 62]. However, it is still not clear currently what kind of acquired behaviors will be transgenerationally inheritable to the descendant generations. More investigations are absolutely needed to uncover the features of acquired behaviors that can be transgenerationally inherited, and thus animal models that can be easily manipulated should be established for this specific purpose to investigate the molecular mechanisms underlying the transgenerational inheritance of acquired behavioral traits.

2.2 Germline transmission in transgenerational inheritance of behavioral traits

An essential question about the inheritance of acquired behavioral traits is how the acquired behaviors that are controlled in the brain are transmitted to the descendant generations after undergoing fertilization and embryonic development. It is interpretable for intergenerational inheritance as the germline cells of the offspring have experienced the environmental stimuli [1, 60], however, it is not the case for transgenerational inheritance. The transgenerational passage of acquired behavioral traits requires the inheritance of epigenetic marks, thus it is the central question for transgenerational inheritance that how the acquired epigenetic marks in the brain involved in the acquired traits, e.g. learning and memory, are transmitted to the offspring. The relationship between the acquired memory in the brain and epigenetic modifications in the germline remains to be uncovered to delineate the mechanistic framework of transgenerational inheritance of learning and memory.

Recent studies on transgenerational epigenetic inheritance of acquired behaviors revealed that transmission of the parental behavioral traits to the descendant is mediated by the changes of epigenetic marks such as DNA methylation, histone posttranscriptional modifications and noncoding RNAs [1, 2, 60]. One of such examples is that the mice experienced low or high maternal care affect the care manner of their offspring [63]. DNA methylation in the genomic regions of the glucocorticoid receptor and the estrogen receptor genes was found to be required for the behavioral inheritance in mice [64–65]. Another study reported that the mice subjected to maternal separation and maternal unpredictable stress displayed behavioral and physiological alterations, involving the changes of miRNA and piRNA in the sperm[66]. Interestingly, these behavioral and physiological alterations were also observed in the offspring of the parental mice experienced such stresses[66].

Together, current findings support the concept that the inheritance of epigenetic marks in the germline is essential for the transgenerational inheritance of acquired behaviors. However, it remains largely unknown what are the mechanistic events linking the behavioral inheritance of learning and memory in the brain to epigenetic inheritance in the germline. More animal models and behavioral paradigms that are stable and easily manipulated await being developed to assess this question.

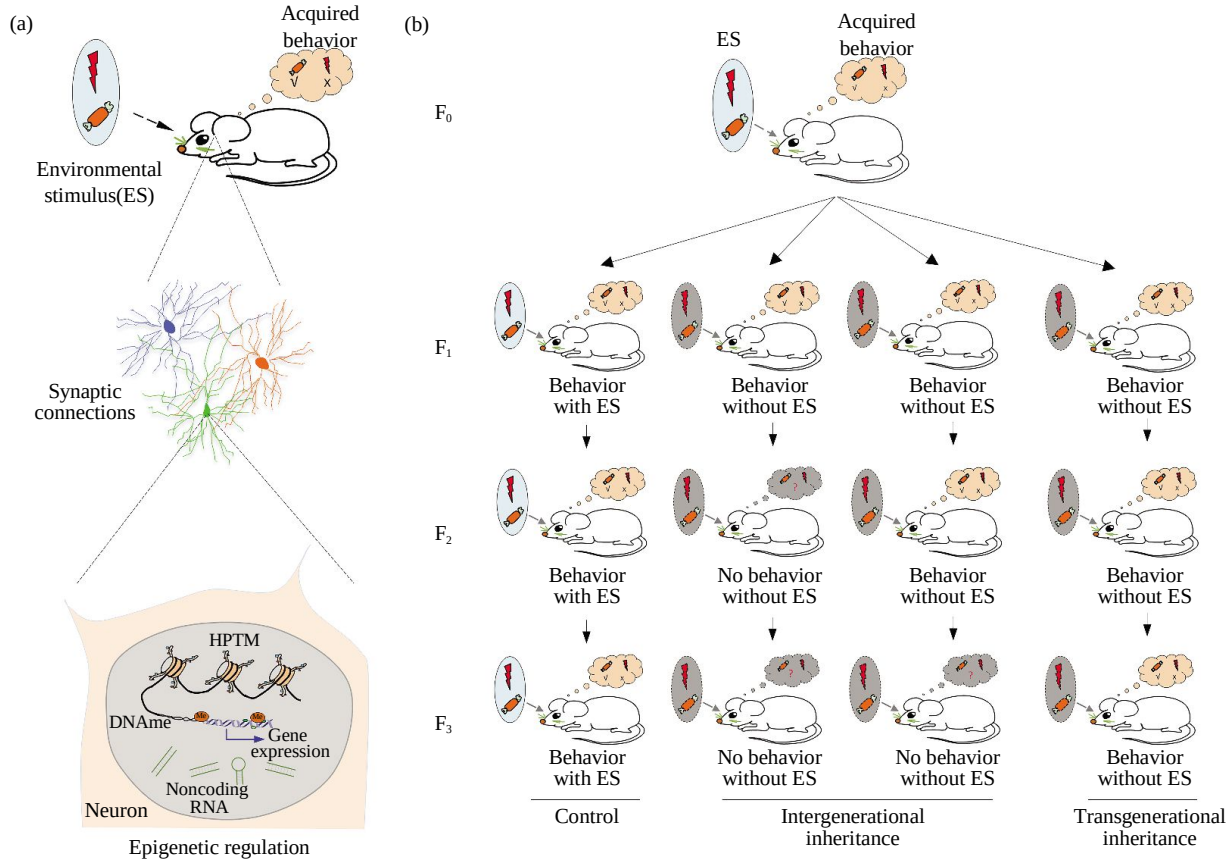

Fig. 1 The molecular basis, neural circuit and transgenerational inheritance of learning and memory

(a) Molecular and neural circuit underlying memory. Memory formation and reconsolidation are linked with the synaptic growth and connections, which are regulated by alterations of epigenetic marks, such as DNA methylation, histone posttranslational modifications (HPTM) and noncoding RNAs. (b) Transgenerational inheritance of acquired behaviors underlying memory. The intergenerational inheritance requires the epigenetic alterations in the germ cells caused by direct effects of the environmental stimulus (ES), whereas the transgenerational inheritance requires epigenetic alterations from the parental generation but not direct effects of the environmental stimulus.

3 Potential *Drosophila* paradigms for studying transgenerational epigenetic inheritance of learning and memory

The molecular and neural circuit mechanisms of learning and memory have been better accepted from the studies using vertebrate behavioral paradigms. However, recent observations that some of the psychiatric diseases such as depression, anxiety and addiction display strong heritable risks to the descendant generations raise the question that defects of learning and memory occur likely not only in one generation but through multiple generations. The molecular basis of behaviors inheritance underlying the memory is largely unknown as there are currently lacking sufficient animal models and feasible behavioral paradigms for this purpose. The current animal models for transgenerational inheritance study mainly rely on the *C. elegens* models for physiological traits (e.g. ageing)[55, 67] and rodent models for maternal behavioral traits [66]. The investigations using these animal models are far from being sufficient for the understanding of transgenerational inheritance of behavioral traits that are based on learning and memory. *Drosophila* is an excellent genetic animal model for studying the mechanistic regulation of development and metabolism [68]. Recent studies on *Drosophila* learning and memory have promoted our understanding of the neurobiology underlying behaviors [9, 11, 69]. Here, we focus on assessing the transgenerational inheritance of learning and memory in *Drosophila* (Figure 2) and propose potential strategies to study the transgenerational inheritance of learning and memory in flies (Figure 3).

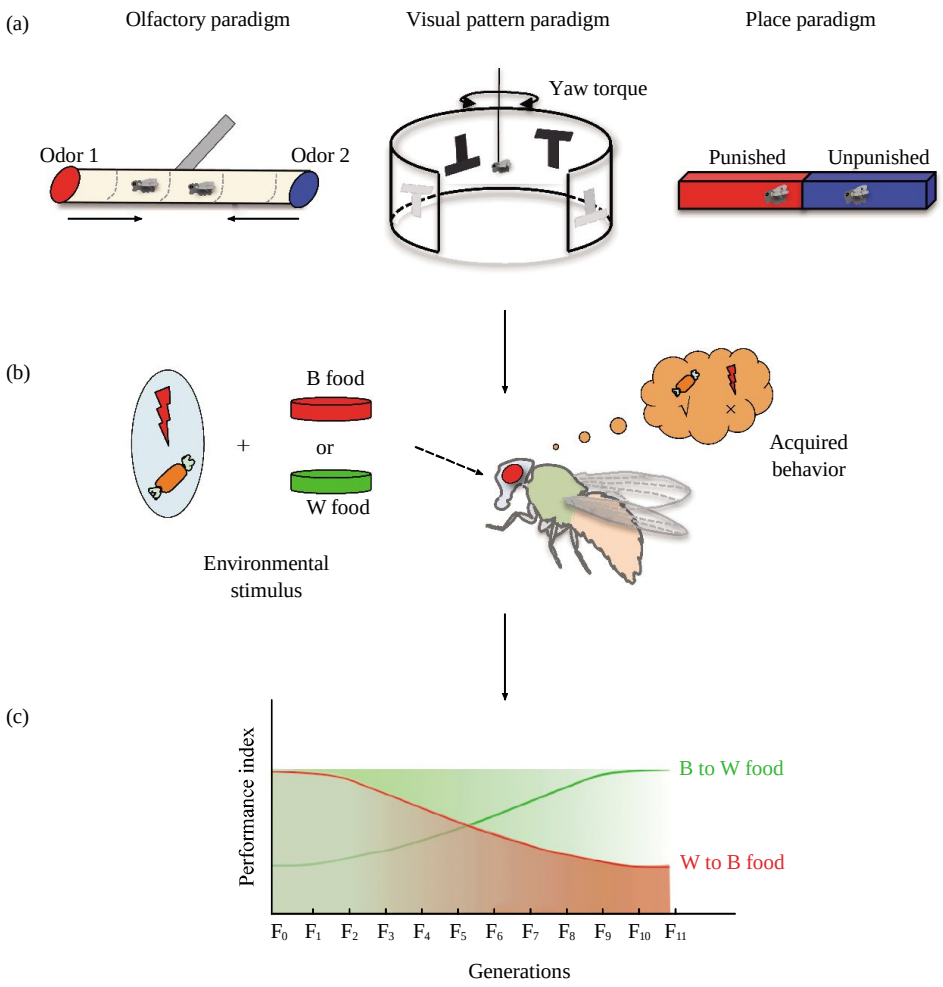

Fig. 2 Strategies for finding out the behaviors that might be transgenerationally inherited in flies

(a) Behavioral paradigms that have been established and used such as olfactory learning, visual pattern learning and place learning paradigms will be tested. (b) The flies fed with Wurzburg food (W food) or Beijing food (B food) will be used for classical conditioning with environmental stimulus (ES). (c) The performance index (PI) of the acquired behaviors will be recorded for at least five generations after food change (from W food to B food or from B food to W food). The acquired behaviors showing gradual changes along with food changes will be used for the further study of transgenerational inheritance.

3.1 Possibility of transgenerational inheritance of learning and memory in *Drosophila*

Recent studies on learning and memory in flies mainly focus on uncovering the genes and neural circuits. As the physiological traits of flies can be transmitted to the descendant generations [70–73], it is tempting to ask whether the behavioral traits of parental flies can also be transmitted to their offspring. There are no reports thus far to investigate transgenerational inheritance of acquired behaviors based on learning and memory in flies. However, it is possible that the flies can not only learn the memory-based behaviors but also transgenerationally transmit the acquired behaviors to the descendant generations.

One evidence to support this hypothesis is that the epigenetic mechanisms have been recently reported to be required for the memory formation in flies[74]. The *Drosophila* euchromatin histone methyltransferase (EHMT) mutant flies displayed decreased non-associative learning and impaired courtship memory [74], implying that histone modifications play important roles in learning and memory in flies. Another study on histone acetylation reported that Tip60 (tat-interacting protein 60), a histone acetyltransferase, is located in the axonal lobe of

mushroom body and required for the courtship learning and intermediate-recall memory [75]. A more recent study showed that the heterochromatin structure regulated by CDK12 (cyclin-dependent kinase 12) is crucial for the courtship learning and memory in flies [76]. These pieces of observations are far from building the circuit mechanisms underlying learning and memory in flies, but it gives rise to the conception that the alterations of epigenetic marks are involved in fly learning and memory, which implies that it is possible for the flies to transmit learning and memory epigenetically to the descendent generations.

Another evidence to support the hypothesis is from a study on visual pattern learning and memory in flies twenty years ago [77]. In this study, Guo and colleagues reported that the environmental conditions such as diet have remarkable effects on the visual flight orientation during classical conditioning [77]. Interestingly, according to the authors' observation the flies fed with Wurzburg food (hereafter referred as W food, with more molasses in the recipe) and Beijing food (hereafter referred as B food, with more sugar in the recipe) show different performance index (PI) of the visual pattern learning in the flight simulator. The flies fed with W food display high PI in the visual pattern learning, whereas the flies fed with B food show low PI. This result indicates that the flies fed with W food is smarter than the flies fed with B food in the visual pattern learning. The most enlightening findings in this study are the following observation that when the flies originally fed with W food were then kept in the B food, they show gradually decreased PI lasting for at least five generations. Reversely, the B food-fed flies show gradually increased PI when changed to the W food. The most interesting finding was the gradual changes of visual pattern learning ability among the descendant generations, implying a gradual change of the molecular and circuit mechanisms based on the learning ability and memory processes. The gradual changes give us another hint that the learning ability of the parent flies transmit to the descendant generations and the memory gradually disappears when the environmental stimuli disappear (such as the W to B food change or B to W food change). More importantly, the molecular and circuitry mechanisms required for the learning ability can be possibly transmitted to the offspring, which is also the memory basis of the offspring.

3.2 Potential strategies for studying the transgenerational inheritance of learning and memory in *Drosophila*

Based on the findings that the learning ability of flies changes according to exogenous stimuli, e.g. varying between W food and B food conditions, here we propose several potential strategies to study the transgenerational inheritance of learning and memory in flies, including the strategies for determining which of the behavioral traits are transgenerationally inheritable (Figure 2) and the strategies for studying how it is regulated (Figure 3).

To study transgenerational inheritance of learning and memory in flies, we first should know what kind of acquired behaviors may be possibly transmitted to the descendant flies. To this end, different behavioral paradigms[78–79](such as olfactory learning, visual pattern learning and place learning paradigms, Figure 2a) can be used to test flies fed on either the W food or the B food (Figure 2b). The visual pattern learning ability is the only example reported to be dependent on diet change, it is important to determine whether there are other learning abilities that show common properties with visual pattern learning ability in response to diet change. As only a single fly can be used in the flight simulator for detection of visual pattern learning, it might be problematic when addressing the question of transgenerational inheritance where one would need more flies to produce offspring. When testing the flies in different behavioral paradigms, the PI of the acquired behaviors will be determined after food change (Figure 2c), from W food to B food or from B food to W food). The acquired behaviors showing gradual changes along with food changes will be used for further investigations.

Following the optimization of behavioral paradigms (Figure 2), strategies for further study of the transgenerational inheritance in *Drosophila* are proposed in Figure 3. The flies fed with the W food are referred as born smart (BS) flies as they display high PI of behavioral learning (Figure 3a); whereas the flies fed with the B food are referred as learned smart (LS) flies as they display low PI of behavioral learning but can eventually get to the high PI after enriched environmental stimuli (ES) such as repeat training schedules(Figure 3d). Based on the BS flies(Figure 3a), the paradigm conditioning/training will be carried out after the food change (from W food to B food)

combined with ES or without ES (Figure 3b). The offspring of the trained flies will then be collected for further paradigm training, and the same training should last for at least three generations. The PI of acquired behaviors of each generation will be determined for further analysis (Figure 3c). The acquired behaviors of parental BS flies that are transmittable to the descendant flies fed on the W food and without training (referred as N(W)) will be the traits for further investigations of the transgenerational inheritance. A comparison of the gene expression profiles, epigenetic marks and neural anatomies between the flies fed on the W food and with training (T(W)) and the flies fed on the W food and without training (N(W)) will give us the mechanistic information of transgenerational inheritance of learning and memory. For the LS flies (Figure 3d), the enriched ES (such as repeat training schedules) similar to that in the mammalian models[52] will be needed for enhanced training of the flies. The flies with enriched ES that show similar PI with the BS flies (referred as LS flies) will be used for further analyses (Figure 3e). The acquired behaviors of the parental LS flies that can be transmitted to the descendant flies fed with B food and without training (referred as N (B)) will be the traits for further investigations of transgenerational inheritance (Figure 3f).

In summary, we suggest *Drosophila* as an animal

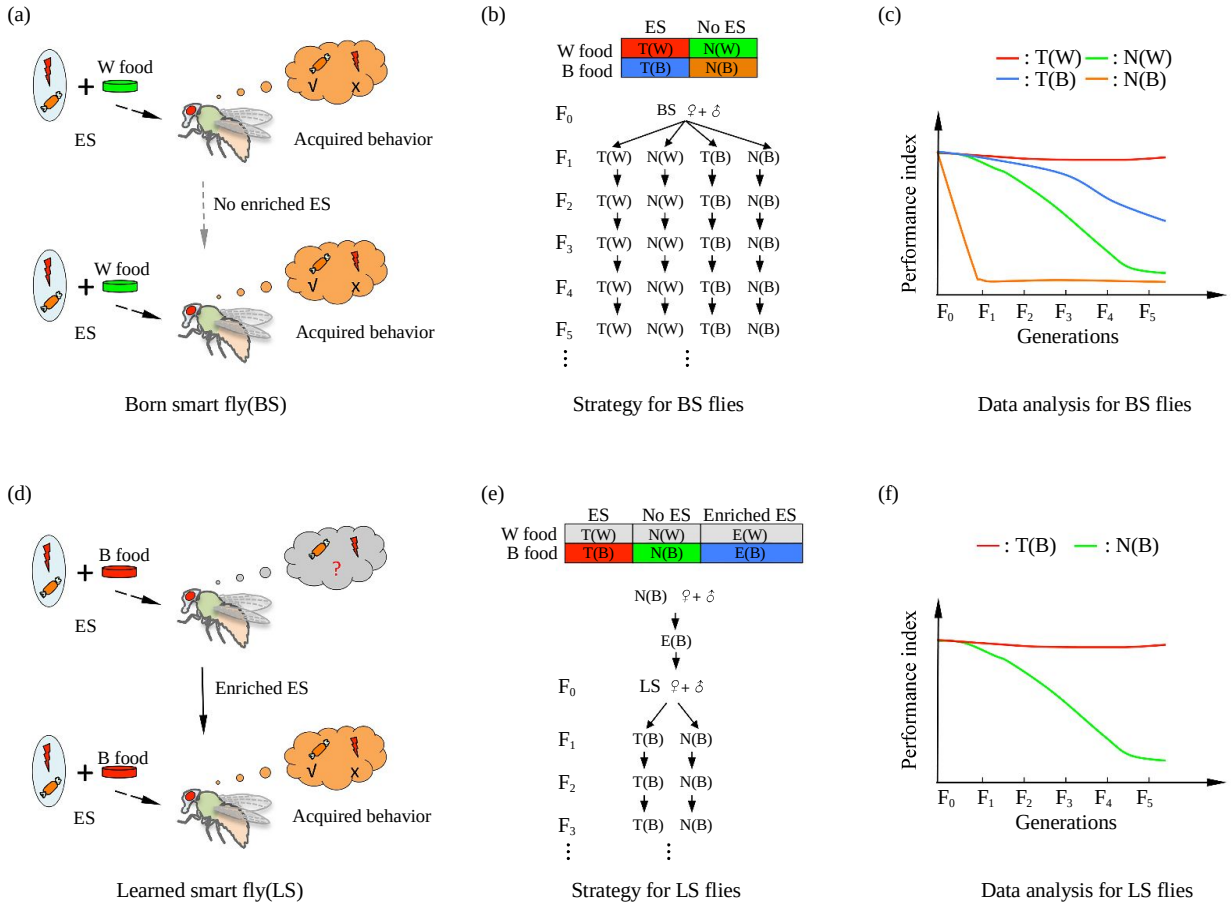

Fig. 3 Strategies for studying the transgenerational inheritance of learning and memory using the born smart (BS) flies and learned smart (LS) flies

(a) The flies fed with W food that display high performance index (PI) in the behavioral paradigms, are referred as born smart (BS) flies. (b) Strategies for studying transgenerational inheritance using BS flies. The BS flies will be trained with or without environmental stimulus (ES and No ES) in W food or B food. The enriched ES represents for enriched environmental stimulus. The offspring of the training flies will then collected for further paradigm training, and this training should last for at least three generations. T(W): Train the W food-fed flies with environmental stimulus (ES); N(W): Do not train the W food-fed flies with ES; T(B): Train the B food-fed flies with ES; N(B): Do not train the B food-fed flies with ES). (c)The performance index (PI) of acquired behaviors of each generation will be recorded for further analysis. (d) The flies fed with B food that display low PI in the behavioral paradigms, are referred as learned smart (LS) flies. (e) Strategies for studying transgenerational inheritance using LS flies. The enriched ES represents for enriched environmental stimulus and will be used to train the flies. The offspring of the training flies will then collected for further paradigm training, and this training should last for at least three generations. T(B): Train the B food-fed flies with environmental stimulus (ES); N(B): Do not train the B food-fed flies with ES; E(B): Train the B food-fed flies with enriched ES. (f)The performance index (PI) of acquired behaviors of each generation will be recorded for further analysis.

model to study the epigenetic mechanisms of transgenerational inheritance of learning and memory. The strategies we proposed here are just general principles, and specific modifications are absolutely needed for each specific experiment when performing paradigms.

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学习和记忆的跨代遗传：果蝇能告诉我们真相吗？*

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摘要 学习记忆是一个获取、储存和再巩固新知识的过程，并以行为作为输出信号．学习记忆是高等生物适应动态环境不可或缺的。学习和记忆能力缺陷会导致精神类疾病，如精神分裂症、抑郁症和阿尔兹海默病等．近年来，有研究发现这些精神类疾病能够遗传给后代，所以以动物模型来研究学习和记忆的跨代遗传机制已经开始．在这篇综述里，首先简要概括了目前有关学习和记忆的分子机制、神经环路和跨代遗传的可能机制；然后，讨论了利用果蝇模型来研究学习和记忆跨代遗传的可能性．最后，我们提供了可能的策略用以揭示果蝇学习和记忆跨代遗传的表观遗传机制．

关键词 跨代遗传，学习和记忆，获得性行为，表观遗传印记，果蝇模型

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