Chapter 19
The Inheritance of an Acquired Taste: Learning and Passing on New Food Odor Preferences in Butterflies

V. Gowri and Antónia Monteiro

Abstract The larvae of butterfly and moth species are often specialist herbivores that feed on very specific plant hosts. However, as a group, they feed on a large diversity of plants. We wondered how diet diversification evolves, i.e., how a specialist acquires the ability and preference to feed on a novel host. We hypothesized that oviposition mistakes may lead larvae to acquire a taste for the novel host, and perhaps even pass on this learned preference to its offspring, facilitating host switches. This chapter details a series of experiments that show this to be the case with larvae of *Bicyclus anynana* butterflies that learn to feed on plant leaves with a novel artificial odor. We also show that the hemolymph of the larvae carries factors that help in the transmission of the learned preference to recipients and to their offspring. Finally, we show that a few genes are differentially expressed in brains of larvae that feed on leaves with and without the odor, but the messenger RNA of these genes are not the epigenetic factors that are inherited by the offspring.

Introduction

Back in the day, Lamarck and Darwin put forth their theories claiming that a characteristic or trait acquired during an organism's lifetime, that changed the behaviour or the morphology of that organism, could be inherited by the offspring (Yawen 2014). Later these theories were opposed by Weissman (Yawen 2015), who proposed the existence of a barrier between somatic cells and germ cells. He proposed that characteristics acquired by the body, in particular by the somatic cells that make most of the body, could not be transferred to the germ cells (sperm or egg), and thus not be inherited. This idea is now accepted by most biologists.

In the past few decades, however, there have been several studies that found gaps in the Weismann barrier. These studies show that organisms can exhibit behavioural

V. Gowri · A. Monteiro (✉)
Department of Biological Sciences, National University of Singapore, Singapore, Singapore
e-mail: antonia.monteiro@nus.edu.sg

G. Witzany (ed.), *Epigenetics in Biological Communication*,
https://doi.org/10.1007/978-3-031-59286-7_19

and morphological changes in response to changes in their environment and can also transmit those acquired traits to the next generation(s). For example, both wild radish plants and water flees can acquire defensive traits and pass them on to the next generation in response to herbivory or predator cues, respectively (Agrawal et al. 1999). In another example, fruit flies can learn to prefer a new odor, and transmit that preference not only to their offspring but also to their grand offspring (Williams 2016).

Nevertheless, how organisms evolve to sense their environment to regulate specific genes and the manner in which acquired traits are passed down to the offspring are still largely unknown. One hypothesis is that they travel via marks deposited on top of genes, in other words via epigenetics—'epi-' meaning on top (Waddington 1968). These non-genetic mutations, which are also known as epigenetic modifications, can modify gene expression and can occur in response to various environmental cues, allowing organisms to alter their phenotype in response to changing conditions more quickly than through genetic mutations (Gowri and Monteiro 2021). Furthermore, many of these epigenetic modifications can be inherited via the germ line and can have long-lasting effects on gene expression profiles. The inheritance of such a mechanism could potentially also pre-adapt offspring to the same novel environment, as experienced by the parents, giving them a fitness advantage.

Although there are several epigenetics studies conducted in plants (Köhler and Springer 2017) and mammals (Skvortsova et al. 2018), that examine how a variety of acquired traits can be transmitted to the next generation, not much has been explored in insects. Herbivorous insects, in particular, are interesting to examine with regards to their larval food preferences because most of them have very specific host plant preferences (Novotny et al. 2012). For example, the lime caterpillar feeds only on lime leaves, the monarch caterpillar feeds on milkweed, and the silk moth caterpillar feeds only on mulberry leaves. Given this diversity of food tastes, it is interesting to ponder how these very specialized preferences have evolved.

Herbivore insects largely rely on their odor senses to find their host plants (Bruce et al. 2005). Preferences for specific hosts are largely due to genetic factors (Singer et al. 1988), but sometimes insects make mistakes, and lay eggs on a different host (Larsson and Ekbom 1995). If the larvae from those eggs survive these mistakes, and grow to adulthood, could these mistakes help larvae learn a preference for a novel host? And could these novel preferences ultimately result in a host-switch? To address these questions, we decided to explore whether insects, and butterfly larvae in particular, are capable of (1) learning a novel host odor within a single generation; (2) pass on this learned odor preference to their offspring; and (3) show an increased preference for the novel odor with successive generations of feeding on the novel host. We detail experiments that found evidence for (1) and (2), but not (3), and also explore the mechanisms for how a novel host odor preference might be learned and inherited at the physiological and molecular levels.

While previous research has found evidence that insects can learn to prefer new odors, most studies couple the odor with either a positive or a negative stimulus, like an electric shock or starvation, that has few parallels in the natural world (Biergans et al. 2016, 2017; Merschbaecher et al. 2012; Dias and Ressler 2014; Williams

2016; Aoued et al. 2020; Deshe et al. 2023). We decided to move away from these traditional odor-learning paradigms and test odor learning in a more natural setting. The new odor is simply fed to the larvae and is not paired with any other stimulus. This recreates a natural oviposition mistake, where a newly hatched larva simply finds itself on top of a novel food plant.

Learning and Transmitting an Odor Preference in *Bicyclus anynana* Butterfly Larvae

Our studies were conducted with *Bicyclus anynana* butterfly larvae. This is a tropical African nymphalid model species that has been reared in the lab for over 40 years. The adult butterflies are fruit feeders and normally feed on mashed bananas in the lab. The larvae feed on a few different grass species but have been feeding on young and tender corn plants ever since they were brought into the lab.

First, we decided to test whether *B. anynana* larvae could learn to prefer a novel food odor within a single generation of feeding on that odor. We scored their host odor choice as soon as they hatched, randomly split them into two groups, and then fed one group with their normal diet of corn leaves, and the other group with leaves coated with a novel banana odor. We then scored odor preference development, over their 20-day larval stage, via a series of odor-choice assays.

Initially, the larvae upon hatching showed an innate avoidance for the banana odor (Gowri et al. 2019). However, after just 5 days of feeding on that odor, this innate avoidance for the banana odor changed to a significant preference for the same. This showed that just a five-day exposure to a new banana odor is enough for the larvae to learn to prefer it. This learned preference became stronger the longer the larvae fed on the odor-coated leaves (Gowri et al. 2019).

To test whether odor-fed parents could transmit the learned odor preferences to their offspring, we mated adults with each other, and tested the innate odor preferences of their naive offspring. Surprisingly, the offspring of banana odor-fed parents preferred the banana odor significantly more than the parents, when they were young hatchlings. This showed that *B. anynana* larvae are capable of learning a novel odor and surviving in a new “host” environment, and also that they transmit these acquired preferences to the next generation (Gowri et al. 2019).

Learned Odor Preferences Last for a Single Generation and Are Reversible

Usually, epigenetic modifications are reversible, i.e., once the novel stimulus is no longer present in the environment, the organism can revert back to its innate preferences or morphology (Abdul et al. 2017). However, there are a few studies that show that acquired preferences can be expressed even though the novel stimulus has been removed from the environment (Waddington 1956; Gibson and Hogness 1996; Sikkink et al. 2014; Remy 2010; Turner 2009). These studies on stabilization of transgenerational inheritance of a learned response appear to depend on the number of generations of exposure to the stimulus, stimulus strength, the stimulus itself, and the model system used. In Lepidoptera, however, there is barely anything known regarding the possibilities of stabilization and maintenance of learned odor preferences via repeated instances of odor exposure across generations. In addition, the epigenetic factors and the molecular mechanisms that are involved in the learning of a novel odor and the inheritance of the same remain unexplored (Fig. 19.1).

We tried to explore if the inheritance of the novel banana odor preference could become stronger with multiple generations of feeding on that odor or become stably inherited and expressed even in the absence of the odor stimulus. A few studies have shown the inheritance of a stable odor preference, even in the absence of the odor stimulus, and these studies are intriguing. For example, when mice were exposed to a novel odor, they learned to prefer the odor and transmitted that learned preference to their offspring, as well as their grand offspring (Dias and Ressler 2014). In the case of the nematode *Caenorhabditis elegans*, when they were exposed to a novel odor for a single generation, they were able to pass on that learned preference to the next generation, but not to their grand offspring. However, when these nematode

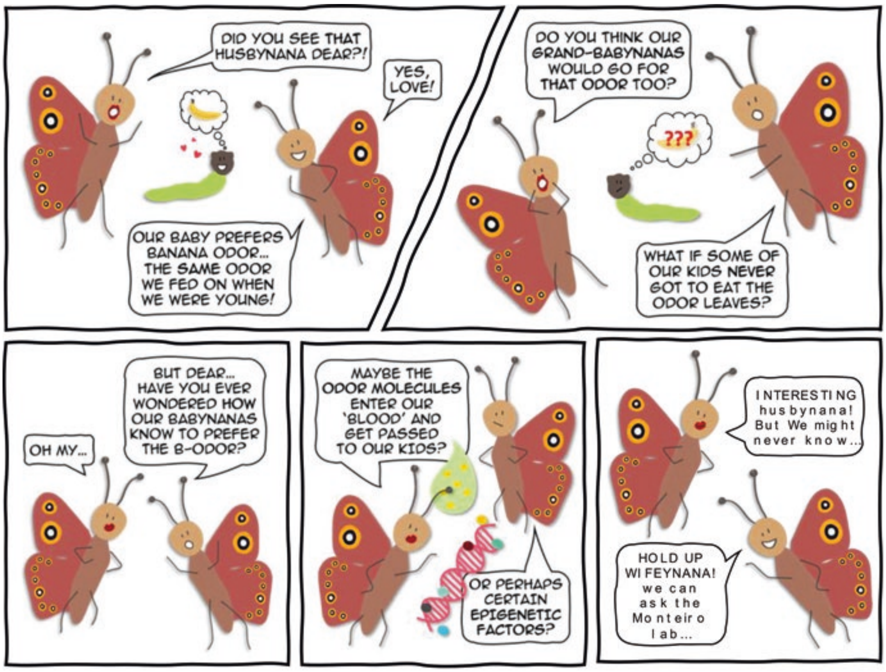

Fig. 19.1 An *anynana* comic. A comic strip depicting the conversation between *wifeynana* and *husbynana* that partially inspired our recent studies. (Image credit: V. Gowri)

worms were reared on the odor continuously for five generations, the next 40 generations showed preferences for the novel odor even though they were no longer reared on that odor (Remy 2010). Hence, we were curious to investigate the effects of long-term odor exposure on *B. anynana* larvae on the persistence and strength of the odor preference.

We reared the larvae on two treatments—control and odor, in which they were fed control leaves and odor-coated leaves. This time we used diluted isoamyl acetate (IAA) as the novel odor instead of a banana flavoring from the supermarket shelf, used in our previous experiments. IAA is a single pure chemical that smells like banana. These larvae fed on their treatments for five generations, after which the odor stimulus was removed from the odor treatment. This tested if the larvae still preferred the odor even though the novel odor stimulus was absent. As in previous experiments, we found that just a single generation of odor exposure was enough to change the innate avoidance for the novel odor to a significant preference for the same. Also, the choices made by the odor treatment larvae and the control treatment larvae were significantly different across generations. However, the preference for the novel odor did not increase across generations (Fig. 19.2; Gowri and Monteiro 2023a). We also found that when the odor stimulus was removed from the leaves, the next generation of larvae immediately lost their preference for the odor and behaved very similarly to the control treatment larvae. This experiment showed a single-generation effect (a parental effect) in the learning of a novel preference, that is reversible. As soon as the parents stopped feeding on the novel odor, their offspring lost the naïve preference for it.

Hemolymph Transfusions Are Able to Transfer an Odor Preference to Naïve Recipient Larvae and to Their Offspring

We determined that *B. anynana* larvae could learn a preference for a novel odor and pass this preference onto the next generation, but we still had no idea how they did this. If a preference develops in the brain of a larva, how is this preference transferred from the brain to the germline of the larva?

To tackle this question, we shifted our focus to the hemolymph. Hemolymph is similar to our blood. It is the circulating fluid in insects (Hillyer and Pass 2020), and could be a vehicle for the transfer of "epigenetic factors" from the brain to the germ line. There are a few studies that show that hemolymph transfusions can alter the phenotype of a recipient to resemble that of the donor (Rodrigues et al. 2010; Wiesner 1991; Otaki 1998; Sondhi 1960). For example, mosquitoes that lay eggs do not look for hosts. So, when hemolymph from egg-laying mosquitoes was transfused to non-egg laying individuals, these recipient mosquitoes stopped seeking hosts (Klowden and Lea 1979). This showed that hemolymph transfusions can induce a behavioural change in the recipient animal.

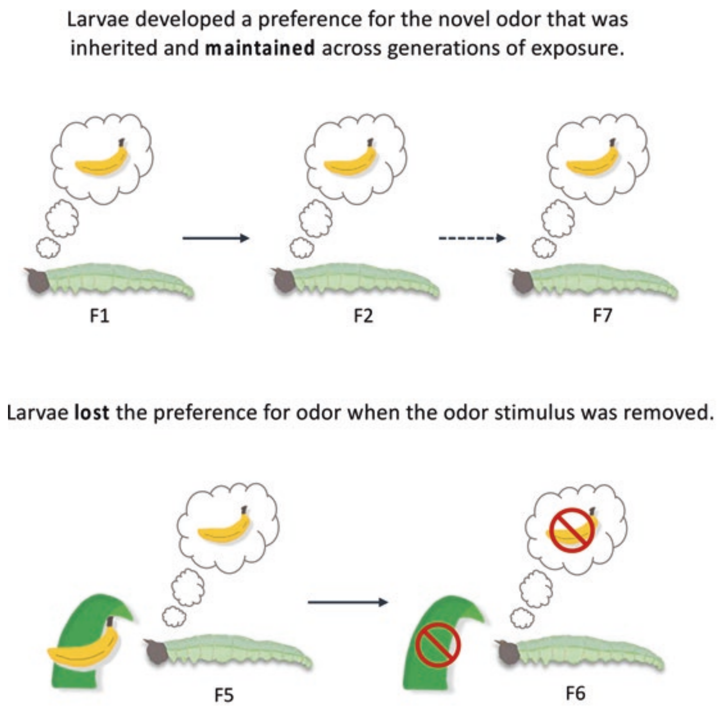

Fig. 19.2 Multi-generational odor-feeding does not increase a larva's preference for the odor nor does it stabilize it, once the odor is removed from the parent's diet. (Image credit: V. Gowri)

We hypothesized that the hemolymph of odor-fed *B. anynana* carried factors that could induce novel odor preferences in naïve recipient individuals and in their offspring. This is a novel hypothesis as there were no studies of this kind, showing any effect of hemolymph transfusions on inducing a 'novel odor' preference in recipients. Moreover, there were no other studies showing that hemolymph transfusions can impact the behavior of the host's offspring.

We extracted hemolymph from two groups of 20-day-old larvae, that were either odor or control-fed, and injected that hemolymph in slightly younger recipient larvae. Donor larvae were monitored (for odor choices) at multiple times throughout their development, and only those that showed a consistent preference for their treatment odor were chosen as donor larvae. We found that before hemolymph transfusions, naïve recipient larvae avoided the novel banana odor. However, after hemolymph transfusion, i.e. just within 24 hours, their innate novel odor avoidance changed to a random choice (Fig. 19.3; Gowri and Monteiro 2023b).

These recipient larvae were then allowed to pupate and develop into butterflies. They mated among themselves and produced offspring, for which innate odor choices were determined as soon as they hatched. We observed that the offspring of larvae that were injected with hemolymph from odor-fed larvae preferred the odor significantly more than offspring of larvae that were injected with hemolymph from

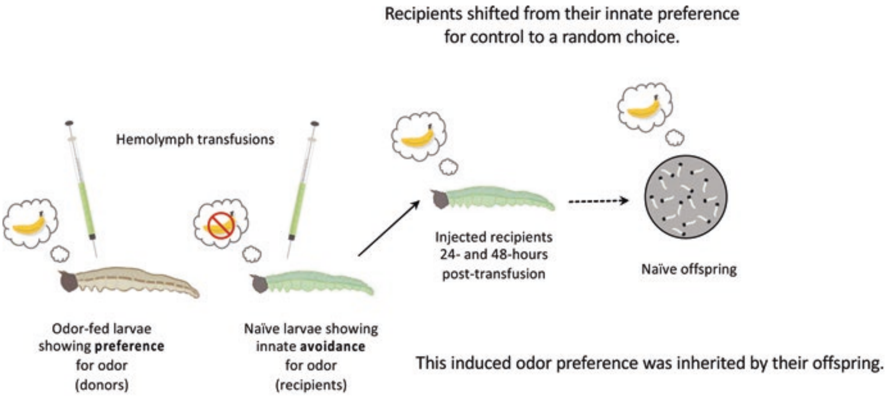

Fig. 19.3 Hemolymph transfusions altered odor preferences in recipient larvae and in their offspring. (Image credit: V. Gowri)

the control treatment larvae. This showed that the hemolymph carries factors (either epigenetic, non-genetic, or the odor molecules themselves) that can change the innate odor preference of a recipient larvae and that of their offspring (Gowri and Monteiro 2023b).

A Series of Genes Are Differentially Expressed in Larval Brains But They Are Not the Heritable Epigenetic Factors

We were keen to investigate the genetic and epigenetic factors that might be involved in the learning of the novel odor in the brain or peripheric nervous system, and in the inheritance of the novel odor preferences. Known heritable epigenetic modifications include DNA methylation, histone modifications, or coding and non-coding RNAs (Gowri and Monteiro 2021; Sengupta et al. 2023). For example, odor-conditioned animals have shown differential expression of olfactory receptor genes and genes related to stress (Brass et al. 2020; Aoued et al. 2019). Differentially expressed short non-coding RNAs such as microRNAs (miRNA) were found in the sperm of older odor-conditioned mice and also in starved worms that showed odor avoidance (Aoued et al. 2020; Deshe et al. 2023). These miRNAs also play a role in odor avoidance and memory formation in fruit flies (Busto et al. 2015). Given these previous studies, we decided to perform gene expression and miRNA analysis in brains and the germline of *B. anynana* subjected to the different odor diets.

We fed larvae on control leaves and odor-coated leaves, and then extracted RNA from their brains when they reached the fifth instar. For a subset of larvae, we let them grow into adults, and then extracted RNA from their oocytes (unfertilized eggs) and spermatophores (sac containing seminal fluid and sperm, that is deposited into the female by the male during mating) of butterflies from both treatments. The RNA samples were then sequenced.

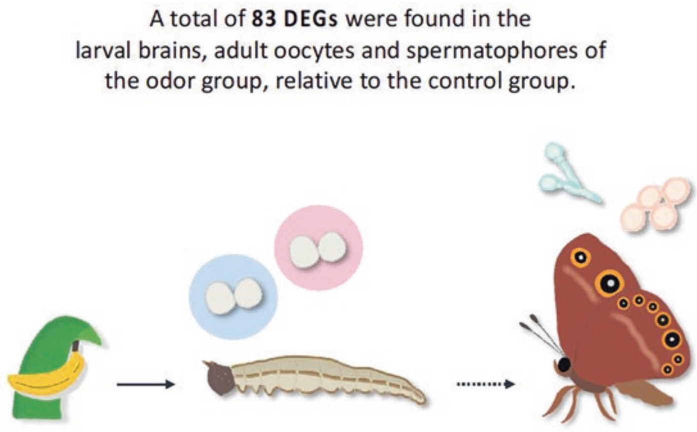

Fig. 19.4 Multiple genes are differentially expressed in the larval brains, and adult germline of *B. anynana* that fed on the different odor diets

A total of 83 genes were found to be differentially expressed across the samples (Fig. 19.4). Not a single gene was shared between tissue types, indicating that mRNA of genes potentially involved in the learning of a novel odor in the brain aren't the factors that are being inherited by the offspring. Of the 83 differentially expressed genes, 23 of them had functions in metabolic activities like host defence, stress sensing, and transcription. Very few genes played known roles in learning and in the olfactory pathway. We also found no differentially expressed miRNAs between treatments in the oocyte samples. However, we found 57 novel miRNAs in these oocytes, upon annotation of the sequenced miRNAs, that were added to databases.

We identified three differentially expressed genes in brains, however, which might be of interest to pursue. The first one was *Pherokine-3* (down-regulated in odor-fed male brains), an insect odorant-binding protein in honey bees (Wang et al. 2013); the second was an organic cation transporter protein-like (down-regulated in odor-fed female brains) shown to play an important role in remote sensing and signaling in *Drosophila* (Engelhart et al. 2020); and the third was the *yellow*-gene (down-regulated in odor-fed male brains), which plays an important role in the eusocial behavior in honey bees, larval foraging in *Drosophila,* and courtship behavior in *B. anynana* (Drapeau et al. 2006; Kucharski et al. 1998; Connahs et al. 2022). Future studies should examine the function of these genes to implicate them further in the process of odor learning and/or odor preference inheritance. As this was the first study of its kind done in lepidopteran larvae, there is much more left to be discovered.

Conclusion

We started by asking how the very specialized preferences of most herbivorous larvae evolve. We wondered whether perhaps this specialization derives from oviposition mistakes made by female butterflies that lead to long-term impacts on diet choices. We hypothesized that if a larva survives this mistake, grows and develops on the new host with a new chemical composition, it might develop a preference for the new host during its lifetime. We also hypothesized that if this preference can be passed down to the next generation, the offspring might benefit, as they start their lives with an immediate odor preference for the novel host, rather than the old host. This could potentially accelerate their growth rate and translate into higher fitness. This mechanism of odor preference learning and inheritance could ultimately facilitate host switching.

We found that larvae of *B. anynana* butterflies, despite displaying a natural avoidance for an artificial banana odor, learned to prefer this odor within days of feeding on it. They also passed this novel odor preference to their offspring. However, the preference did not increase with successive generations of odor feeding, and naïve larvae immediately lost the preference once the odor was removed from their parent’s diet. This odor-learning and inheritance mechanism is, thus, a single-generation parental effect. This flexibility and reversibility might be advantageous to allow larvae to survive future oviposition mistakes made by their mothers, potentially promoting further diet diversification. We propose that this learning mechanism can facilitate host switches, but that genetic changes may ultimately need to stabilize new acquired preferences.

We also found that the hemolymph contains factors that can induce a preference for a novel odor in naïve recipients as well as their offspring. We suspect that this might be the chemical odorant molecule itself. The volatile compound might enter the hemolymph of the larvae through the gut. Gas chromatography-mass spectrometry (GC-MS) can be performed on hemolymph to test this hypothesis. Alternatively, the hemolymph might contain epigenetic factors, perhaps generated in the brain after learning took place, so sequencing the RNA in the hemolymph also might give us some leads. Further, in addition to RNA sequencing, DNA methylation should also be tested in future, both in brains and in the germline, as these methylation marks can be inherited. These molecular candidates should then be tested at the functional level to examine their role in either odor preference learning, or odor preference inheritance in butterfly larvae.

Acknowledgements We thank Shen Tian for helping to analyze miRNAs in the oocytes of *B. anynana*. This work was supported by a Yale-NUS PhD scholarship to V. Gowri and by the Ministry of Education (MOE), Singapore award MOE2018-T2-1-092 and the National Research Foundation, Singapore under its Investigatorship programme (award NRF-NRFI05-2019-0006).

References

Abdul QA, Yu BP, Chung HY, Jung HA, Choi JS (2017) Epigenetic modifications of gene expression by lifestyle and environment. Arch Pharm Res 40:1219–1237

Agrawal AA, Laforsch C, Tollrian R (1999) Transgenerational induction of defences in animals and plants. Nature 401(6748):60–63

Aoued HS, Sannigrahi S, Doshi N, Morrison FG, Linsenbaum H, Hunter SC, Walum H, Baman J, Yao B, Jin P (2019) Reversing behavioral, neuroanatomical, and germline influences of intergenerational stress. Biol Psychiatry 85(3):248–256

Aoued HS, Sannigrahi S, Hunter SC, Doshi N, Sathi ZS, Chan AWS, Walum H, Dias BG (2020) Proximate causes and consequences of intergenerational influences of salient sensory experience. Genes Brain Behav 19(4):e12638

Biergans SD, Claudianos C, Reinhard J, Galizia CG (2016) DNA methylation adjusts the specificity of memories depending on the learning context and promotes relearning in honeybees. Front Mol Neurosci 9:82

Biergans SD, Claudianos C, Reinhard J, Galizia CG (2017) DNA methylation mediates neural processing after odor learning in the honeybee. Sci Rep 7(1):43635

Brass KE, Herndon N, Gardner SA, Grindstaff JL, Campbell P (2020) Intergenerational effects of paternal predator cue exposure on behavior, stress reactivity, and neural gene expression. Horm Behav 124:104806

Bruce TJ, Wadhams LJ, Woodcock CM (2005) Insect host location: a volatile situation. Trends Plant Sci 10:269–274

Busto GU, Guven-Ozkan T, Fulga TA, Van Vactor D, Davis RL (2015) microRNAs that promote or inhibit memory formation in *Drosophila melanogaster*. Genetics 200(2):569–580

Connahs H, Tan EJ, Ter YT, Dion E, Matsuoka Y, Bear A, Monteiro A (2022) The yellow gene regulates behavioural plasticity by repressing male courtship in Bicyclus anynana butterflies. Proc R Soc B 289(1972):20212665

Deshe N, Eliezer Y, Hoch L, Itskovits E, Bokman E, Ben-Ezra S, Zaslaver A (2023) Inheritance of associative memories and acquired cellular changes in *C. elegans*. Nat Commun 14(1):4232

Dias BG, Ressler KJ (2014) Parental olfactory experience influences behavior and neural structure in subsequent generations. Nat Neurosci 17(1):89–96

Drapeau MD, Albert S, Kucharski R, Prusko C, Maleszka R (2006) Evolution of the Yellow/Major Royal Jelly Protein family and the emergence of social behavior in honey bees. Genome Res 16(11):1385–1394

Engelhart DC, Azad P, Ali S, Granados JC, Haddad GG, Nigam SK (2020) *Drosophila* SLC22 orthologs related to OATs, OCTs, and OCTNs regulate development and responsiveness to oxidative stress. Int J Mol Sci 21(6):2002

Gibson G, Hogness DS (1996) Effect of polymorphism in the *Drosophila* regulatory gene Ultrabithorax on homeotic stability. Science 271(5246):200–203

Gowri V, Monteiro A (2021) Inheritance of acquired traits in insects and other animals and the epigenetic mechanisms that break the Weismann barrier. J Dev Biol 9(4):41

Gowri V, Monteiro A (2023a) Acquired preferences for a novel food odor do not become stronger or stable after multiple generations of odor feeding in Bicyclus anynana butterfly larvae. Ann N Y Acad Sci 1531:1–11

Gowri V, Monteiro A (2023b) Hemolymph transfusions transfer heritable learned novel odor preferences to naive larvae of Bicyclus anynana butterflies. BioRxiv. https://doi.org/10.1101/2023.12.20.572719. 2012–2023

Gowri V, Dion E, Viswanath A, Piel FM, Monteiro A (2019) Transgenerational inheritance of learned preferences for novel host plant odors in *Bicyclus anynana* butterflies. Evolution 73(12):2401–2414

Hillyer JF, Pass G (2020) The insect circulatory system: structure, function, and evolution. Annu Rev Entomol 65:121–143

Klowden MJ, Lea AO (1979) Humoral inhibition of host-seeking in Aedes aegypti during oöcyte maturation. J Insect Physiol 25(3):231–235

Köhler C, Springer N (2017) Plant epigenomics—deciphering the mechanisms of epigenetic inheritance and plasticity in plants. Genome Biol 18:132.

Kucharski R, Maleszka R, Hayward DC, Ball EE (1998) A royal jelly protein is expressed in a subset of Kenyon cells in the mushroom bodies of the honey bee brain. Naturwissenschaften 85(7):343–346

Larsson S, Ekbom B (1995) Oviposition mistakes in herbivorous insects: confusion or a step towards a new host plant? Oikos 72(1):155–160

Merschbaecher K, Haettig J, Mueller U (2012) Acetylation-mediated suppression of transcription-independent memory: bidirectional modulation of memory by acetylation. PLoS One 7(9):e45131

Novotny V, Miller SE, Hrcek J, Baje L, Basset Y et al (2012) Insects on plants: explaining the paradox of low diversity within specialist herbivore guilds. Am Nat 179:351–362

Otaki JM (1998) Color-pattern modifications of butterfly wings induced by transfusion and oxyanions. J Insect Physiol 44(12):1181–1190

Remy J-J (2010) Stable inheritance of an acquired behavior in *Caenorhabditis elegans*. Curr Biol 20(20):R877–R878

Rodrigues J, Brayner FA, Alves LC, Dixit R, Barillas-Mury C (2010) Hemocyte differentiation mediates innate immune memory in *Anopheles gambiae* mosquitoes. Science (New York, NY) 329(5997):1353–1355

Sengupta T, Kaletsky R, Murphy CT (2023) The logic of transgenerational inheritance: timescales of adaptation. Annu Rev Cell Dev Biol 39:45–65

Sikkink KL, Reynolds RM, Ituarte CM, Cresko WA, Phillips PC (2014) Rapid evolution of phenotypic plasticity and shifting thresholds of genetic assimilation in the nematode *Caenorhabditis remanei*. G3 (Bethesda) 4(6):1103–1112

Singer MC, Ng D, Thomas C (1988) Heritability of oviposition preference and its relationship to offspring performance within a single insect population. Evolution 42:977–985

Skvortsova K, Iovino N, Bogdanović O (2018) Functions and mechanisms of epigenetic inheritance in animals. Nat Rev Mol Cell Biol 19:774–790

Sondhi KC (1960) Selection for a character with a bounded distribution of phenotypes in *Drosophila subobscura*. J Genet 57(2):193–221

Turner BM (2009) Epigenetic responses to environmental change and their evolutionary implications. Philos Trans R Soc B Biol Sci 364:3403–3418

Waddington CH (1956) Genetic assimilation of the Bithorax phenotype. Evolution 10(1):1–13

Waddington CH (1968) Towards a theoretical biology. Nature 218(5141):525–527

Wang Z-L, Wang H, Qin Q-H, Zeng Z-J (2013) Gene expression analysis following olfactory learning in *Apis mellifera*. Mol Biol Rep 40:1631–1639

Wiesner A (1991) Induction of immunity by latex beads and by hemolymph transfer in *Galleria mellonella*. Dev Comp Immunol 15(4):241–250

Williams ZM (2016) Transgenerational influence of sensorimotor training on offspring behavior and its neural basis in *Drosophila*. Neurobiol Learn Mem 131:166–175

Yawen Z (2014) Charles Darwin's theory of pangenesis. In: Embryo project encyclopedia. Arizona State University, School of Life Sciences, Center for Biology and Society, Tempe

Yawen Z (2015) The germ-plasm: a theory of heredity (1893). In: Weismann A (ed) Embryo project encyclopedia. Arizona State University, School of Life Sciences, Center for Biology and Society, Tempe