

OIKOS

Research Article

Nectar concentrations of biogenic amines affect bumble bee behavior in a dose-dependent manner

Marta Barberis¹✉, Daniele Calabrese^{2,3}, David Baracchi⁴, Laura Bortolotti⁵, Flavia Di Cesare^{4,6}, Rosa Ranalli^{5,7}, Laura Zavatta^{5,8}, Massimo Nepi^{2,3}, Marta Galloni^{1,3} and Gherardo Bogo⁵

¹Department of Biological, Geological, and Environmental Sciences, University of Bologna, Bologna, Italy

²Department of Life Sciences, University of Siena, Siena, Italy

³National Biodiversity Future Centre (NBFC), Palermo, Italy

⁴Department of Biology, University of Florence, Sesto Fiorentino, Italy

⁵CREA Research Centre for Agriculture and Environment, Bologna, Italy

⁶Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy

⁷Department of Biotechnology and Biosciences, University of Milano Bicocca, Milano, Italy

⁸Department of Agriculture and Food Sciences, University of Bologna, Bologna, Italy

Correspondence: Marta Barberis (marta.barberis2@unibo.it)

Oikos

2025: e11563

doi: 10.1002/oik.11563

Subject Editor:

Paulo R. Guimaraes

Editor-in-Chief:

Paulo R. Guimaraes

Accepted 31 July 2025



While foraging, pollinators encounter a wide array of molecules, some of which may influence their internal states and behaviors. Among these, biogenic amines – nitrogenous compounds that serve as essential neuromodulators in animals – have only recently been detected in floral nectar. As a result, little is known about the effects of nectar concentrations of these compounds on pollinators. In this study, we tested how two key biogenic amines, octopamine and its precursor tyramine, affect bumble bee behavior by examining: 1) consumption and survival, 2) locomotion, and 3) feeding behavior. Our findings show a preference for low concentration of octopamine (0.1 mM) and a dose-dependent effect on flight behavior. Specifically, a concentration equal to 0.1 mM of octopamine reduces the bee's motivation to fly, whereas a concentration of 1 mM increases flight duration. Tyramine has no significant effect on any of the behaviors tested. Our results demonstrate that nectar-borne biogenic amines have the potential to influence various bumble bee behaviors critical to flower visitation.

Keywords: *Bombus terrestris*, feeding behavior, locomotion, neuroactive nectar, octopamine, tyramine

Introduction

Since the 1970s, hundreds of secondary metabolites have been found in floral nectar (Baker and Baker 1986, Palmer-Young et al. 2019, Barberis et al. 2023a). They were shown to affect pollinator behavior in a variety of ways, challenging the traditional view of nectar as a simple food reward or attractant (Stevenson et al. 2017, Nepi et al. 2018, Bogo et al. 2021, Barberis et al. 2024). Among these nectar components, some



www.oikosjournal.org

© 2025 The Author(s). Oikos published by John Wiley & Sons Ltd on behalf of Nordic Society Oikos.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

have gained attention for their neuromodulatory effects on pollinator nervous systems (Nepi 2014, Mustard 2020). Whereas some, such as alkaloids, have been known since the 1980s, and are nowadays well recognized for affecting pollinator behavior at nectar concentrations (Wright et al. 2013, Thomson et al. 2015, Baracchi et al. 2017a, Bogo et al. 2019, 2024, Carlesso et al. 2021), others, such as biogenic amines, have only recently been discovered in floral nectar. The first study reporting biogenic amines in nectar was published by Muth et al. (2022), showing the presence of this chemical class in 15 plant species covering six plant orders. These chemicals are represented by nitrogenous compounds known to function as neuromodulators in vertebrates and invertebrates (Orchard 1982, Roeder 2000, Blenau and Baumann 2001, Scheiner et al. 2006, Farooqui 2012). To date, tyramine and octopamine are the only biogenic amines reported in floral nectar, with the highest concentrations reported in the species *Citrus x meyeri* and *Echium vulgare* (Muth et al. 2022, Barberis et al. 2023b, 2023c).

Tyramine and octopamine are structurally related to the vertebrate adrenaline and noradrenaline, with which they share similar physiological roles, suggesting an early evolutionary origin of the adrenergic/octopaminergic/tyraminer-gic system (Roeder 2005, Muth et al. 2023). The fight-or-flight response, a prompt adaptation to energy-demanding situations (Roeder 2005), is only one of several examples that can be made to show the involvement of such a system in ruling insect behavior. Octopamine and tyramine are products of the decarboxylation of the amino acid tyrosine and even though tyramine represents the biological precursor of octopamine, they are considered to act as independent neuromodulators in insects, through their distinct binding profiles to G protein-coupled receptors (Roeder 2005, Kononenko 2009). Compounds that can activate or inhibit G proteins in neurons can affect pollinator behavior both in a short- and long-term way (Mustard 2020). For this reason, even before their discovery in floral nectar, several studies had already provided evidence of the effects octopamine and tyramine have on insects. In pollinators, for example, they have been shown to modulate motivation and general activity, sensitization, locomotion, phototaxis, reward-seeking, learning, memory and social communication (Barron et al. 2007, Baracchi et al. 2020, Peng et al. 2020, Finetti et al. 2021, Muth et al. 2022). Octopamine, in particular, also acts as a value system and instructs the brain on the relevance of external events (Hammer 1993, Giurfa 2006). In foraging behavior, octopamine has been shown to substitute for the reinforcement function in appetitive associative learning (Giurfa 2006, Perry and Barron 2013, Muth et al. 2023). Indeed, in honey bees, the octopaminergic neuron VUMmx1 substitutes for sucrose reinforcement in olfactory PER conditioning. Pairing an odor with electrophysiological stimulation of VUMmx1, which releases octopamine, or with octopamine injections enhances PER learning (Hammer 1993, Hammer and Menzel 1998), while silencing octopaminergic receptor expression in the antennal lobe blocks it (Farooqui et al. 2003). Yet, most studies used biogenic amine concentrations far exceeding natural

nectar levels (Muth et al. 2022), and administered them via injection or directly into the haemolymph or nervous system, leaving open the question of whether ingested octopamine and tyramine can be absorbed, cross the blood-brain barrier, and exert neuroactive effects.

In this study, we investigated the effects of diets enriched with tyramine and octopamine at concentrations corresponding to their natural extremes (0.1 mM and 1 mM) found in the nectar of *Echium vulgare* Linnaeus (Barberis et al. 2023b, 2023c). Specifically, we conducted a series of behavioral experiments to study how these compounds influence consumption and survival, locomotion and feeding behavior in the model bee species *Bombus terrestris*.

Material and methods

Treatment solutions

We tested the following artificial nectar solutions: a control solution (hereafter Control) composed of distilled water and sucrose 50% (w/v), and the same solution enriched with either octopamine hydrochloride (OA) or tyramine (TA) at concentrations of 0.1 mM or 1 mM. Therefore, a total of five different treatment diets (namely: Control, OA 0.1, OA 1, TA 0.1, TA 1) were tested in all the experiments. These concentrations were chosen as they represent the upper and lower range of natural nectar concentrations found in previous studies conducted on the species *Echium vulgare* (Barberis et al. 2023b, 2023c). According to standard practice in experiments with biogenic amines, ascorbic acid was added to all solutions at a concentration of 1.75 mg ml⁻¹ to minimize oxidation of the biogenic amines (Scheiner et al. 2002, Linn et al. 2020). Solutions were stored at 5 ± 1°C and prepared twice a week. In both experiment 1 and experiment 2, bees were provided with fresh solution every other day. All chemicals were purchased from Sigma-Aldrich.

Model species and experimental conditions

Bumble bee *Bombus terrestris* colonies were purchased from Bioplanet srl, Cesena (Italy), maintained at 24 ± 1°C and 43 ± 5% RH in continuous darkness and fed ad libitum with fresh-frozen honeybee-collected pollen (purchased from L'ape e il fiore di Cassanelli Claudio, Sant'Agata Bolognese, Italy) and sugar syrup for three days before the start of the experiment. Sugar syrup was provided by Bioplanet srl, Cesena (Italy), and it came inside a tank underneath each bumblebee colony. Each colony contained approximately 60 workers, brood at all developmental stages and a laying queen. In all experiments, we excluded very small and very large worker bees, as well as newly emerged and old individuals (Bogo et al. 2024).

Consumption and survival (experiment 1)

Following the method described by Bogo et al. (2024), a total of 169 worker bees were collected from four colonies (each colony being a replicate) under red light and individually transferred into Nicot cages (each treatment represented by a

minimum of 33 individual bees) (Supporting information). Nicot cages were purchased from Lega Italy (Faenza, Italy). Once caged, selected bees were individually weighed and divided into weight classes to ensure even distribution among treatment groups and minimize size-related bias. Worker bees were then acclimatized to the test conditions with a 50% (w/v) sucrose solution provided ad libitum by means of 2.5 ml tipless syringes functioning as feeders. The acclimatization period lasted 24 ± 2 h. The syringes were then replaced with new syringes containing the treatment diets. For 21 consecutive days, the syringes were weighed daily at the same time interval (from 9:00 a.m. to 12:00 p.m.), to calculate bumble bee consumption and, if necessary, refilled with fresh solution. The solutions were administered ad libitum. To account for evaporation rate, five syringes of each test solution were arranged in empty Nicot cages. Before- and after-consumption weights were noted to calculate bee consumption, then corrected for evaporation. Similarly, mortality was recorded daily. Worker bees were maintained in a dark climate room under stable conditions of temperature ($24 \pm 1^\circ\text{C}$) and relative humidity ($43 \pm 5\%$ RH).

Locomotion (experiment 2)

The method described by Bogo et al. (2019) was adopted with minor modifications. A total of 75 individuals were collected from three colonies (each colony being a replicate) under red light. The bees were individually marked with different water-based colors (Uni Posca, Mitsubishi pencil) for individual tracking over time and transferred in groups of 5 into 15 experimental cages per colony (three cages per treatment) (Supporting information). Cages were plastic net cylinders (length = 25 cm, diam. = 16 cm) mounted horizontally with the ends closed by transparent plastic lids (Supporting information). They were maintained at room temperature with a 14:10 h L:D cycle.

Once a day, we recorded the amount of solution consumed by weighing the syringes. Before- and after-consumption weights were noted to calculate bee consumption, then corrected for evaporation. The amount was then divided by the number of live bees in each cage to obtain the mean individual daily consumption. Behavior was recorded twice a day (once in the morning and once in the afternoon). At each event, each bee (individually marked for tracking) was observed for one minute, and the duration of different behavioral categories (i.e. walking, feeding, flying and resting) was recorded by means of a vocal recorder. The frequency of each behavior was then calculated, as well as the percentage of time spent in a dynamic (flying + walking + feeding) or a static behavior (staying still). Consumption, survival and behaviors were recorded until the end of the experiment, which lasted nine days.

Feeding behavior (experiment 3)

A total of 60 individuals (12 per treatment diet) (Supporting information) were individually collected from three colonies, transferred into a Falcon tube, and tested for their fine feeding responses according to the protocol designed by Ma et al.

(2016). Falcon tubes were purchased from Di Giovanni Srl. The transfer of individuals into Falcon tubes was conducted under red light, while the administration of the solution was performed under natural light. Bumblebees transferred into the Falcon tubes were not harnessed, but free to approach the solution. Treatment solutions were dispensed using a 100 μl microcapillary tube to individuals that had been starved for 2–4 h. Their behavior was recorded on digital videos (10 FPS) by means of a digital microscope (Dino-Lite AM3113T, AnMo Electronics Corporation) to analyze the fine structure of feeding behavior by continuously scoring the position of the proboscis for two minutes after the first contact with the solution. Video recordings were analyzed using the event logging software Mangold Interact (Mangold International). The three behaviors taken into account were: 1) drinking, when distinct proboscis bouts (i.e. extension of the glossa) indicated that the worker was actively sucking the solution; 2) tasting, when the bee explored and searched for the solution with the proboscis extended, with or without contact with the solution; 3) proboscis retrieving, when the proboscis was stowed under the head, the bee was out of sight, or stopped any attempt to approach the feeder. The number of proboscis bouts was counted and recorded during each drinking phase, and the frequency of each behavior within a single two-minute recording was calculated a posteriori. The volume of solution consumed was also recorded by measuring the pre- and post-consumption lengths of the solution in the microcapillary tube using a digital caliper during a ten-minute exposure. Again, before- and after-consumption weights were subsequently corrected for evaporation.

Statistical analyses

Consumption and survival (experiment 1)

To evaluate differences in feed consumption between bumble bee workers fed different treatment diets, we fitted a generalized linear mixed-effect model (GLMM) with a Gamma error structure-inverse-link function. We set consumption as the response variable and treatment as explanatory variable. Treatments comprised the control and the four sugar solutions enriched with tyramine and octopamine at two different concentrations (0.1 mM or 1 mM). We included bee ID nested within colony ID as a random factor to account for individual autocorrelation. To compare the survival of bees fed different solutions, a log-rank Kaplan–Meier (K–M) survival analysis was carried out by means of the *ggsurvplot* function of the ‘survminer’ package (Kassambara et al. 2021).

Locomotion (experiment 2)

To evaluate differences in flight behavior induced by different treatment diets, we fitted a two-part mixed effect model for semi-continuous zero-inflated data. The model allowed us to assess 1) the effects of the treatment diets on the likelihood of bees engaging in flight versus non-engaging, and 2) the effects of the treatment diets on the duration of flight, when flight occurred. The model was built using the *mixed_model* function of the ‘GLMMadaptive’ package (Rizopoulos 2022),

using a *hurdle.lognormal* distribution as distribution family. Flight duration was set as the independent variable, treatment as a fixed effect, and bee ID nested within colony ID was set as random effect to account for individual autocorrelation. To evaluate the influence of the treatment diets on bee dynamism, we created a two-vector variable comprehensive of the proportion of time spent engaging in static versus dynamic behavior. We then built a GLMM model with a binomial error structure-logit-link function where such two-vector variable was set as response variable, whilst treatment was set as explanatory variable. We included once again the bee ID nested with colony ID as a random effect to account for individual and colony autocorrelation. Individual consumption was calculated by dividing the total daily consumption by the number of bees present in each cage (5). A GLMM was built with a Gamma error structure-inverse-link function. Again, consumption was set as the response variable and treatment as the explanatory variable. We included colony ID as a random effect to account for non-independence between individuals belonging to the same colony.

Feeding behavior (experiment 3)

For the analysis of the data on gustatory response we built either a linear mixed model (LMM) or a generalized linear mixed model (GLMM) with a Gamma error structure-log-link function on the arcsin-transformed percentage of the duration of each behavioral state (drinking, tasting, losing interest). For the data on the frequency of each behavioral state and the number of bouts we built generalized linear mixed models (GLMMs) with a Poisson error structure-link function. An additional GLMM with a Gamma error structure-log-link function was also built for the consumption data. In all these models, each variable was set as a dependent variable, the treatment diet was included as a fixed factor and

bee ID nested within colony ID was included as a random factor to account for individual variability.

All generalized linear mixed models were built using the *glmmPQL* function of the R package 'nlme' (Pinheiro et al. 2022), then followed by a pairwise contrast effect estimation performed through the R package 'emmeans' (Lenth 2021). All statistics were performed using RStudio software (ver. 4.0.2) with the α -error set at 0.05.

Results

Consumption and survival (experiment 1)

Bees fed OA 0.1 diets consumed more solution than both those fed Control (Control:OA 0.1, $t_{163} = 3.076$, $p = 0.021$) and those fed OA 1 diets (OA 0.1:OA 1, $t_{163} = -3.944$, $p = 0.001$). Bees fed tyramine-enriched diets did not differ from control bees (Fig. 1, Supporting information).

No significant difference in survival time was found among bees fed different treatment diets (log-rank, $p = 0.730$; Supporting information).

Locomotion (experiment 2)

Worker bees fed TA 0.1 and TA 1 diets consumed significantly less solution than those fed Control (Control:TA 0.1, $t_{157} = -2.872$, $p = 0.037$; Control:TA 1, $t_{157} = -4.384$, $p < 0.001$). Also, bees fed TA 0.1 diets consumed significantly less than those fed OA 0.1 diets (OA 0.1:TA 0.1, $t_{157} = -3.082$, $p = 0.020$). (Fig. 2, Supporting information).

Bees fed OA 0.1 engaged a significantly lower number of times in flight than those fed Control (OA 0.1, $z = 2.790$, $p = 0.005$) (Fig. 3a, Supporting information). However, when engaging in flight, bees fed OA 1 flew for longer than those fed Control (OA 1, $z = 2.158$, $p = 0.031$) (Fig. 3a, Supporting

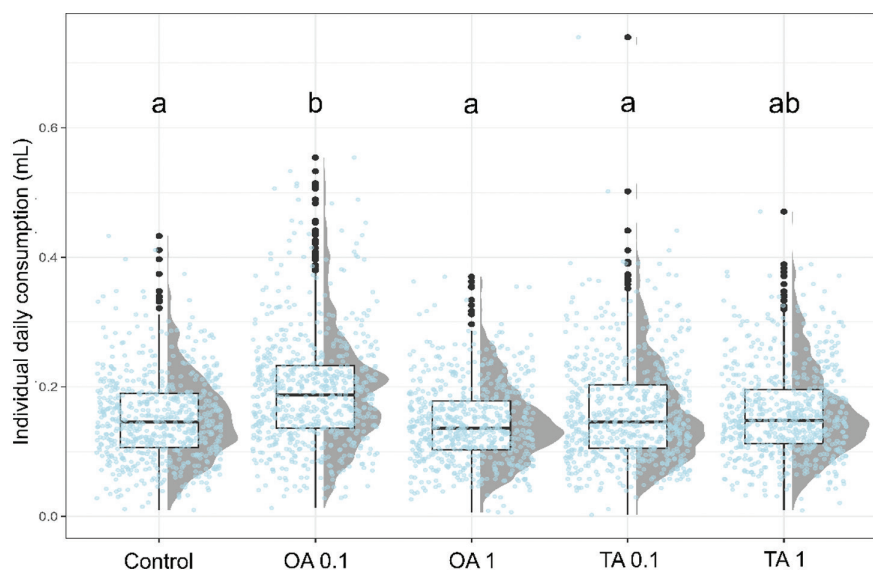


Figure 1. Individual daily consumption (ml) of the five treatment diets recorded for worker bumble bees individually caged in Nicot cages (experiment 1). Different letters indicate significant differences among treatments. Control, $n = 680$; OA 0.1, $n = 680$; OA 1, $n = 660$; TA 0.1, $n = 660$; TA 1, $n = 680$.

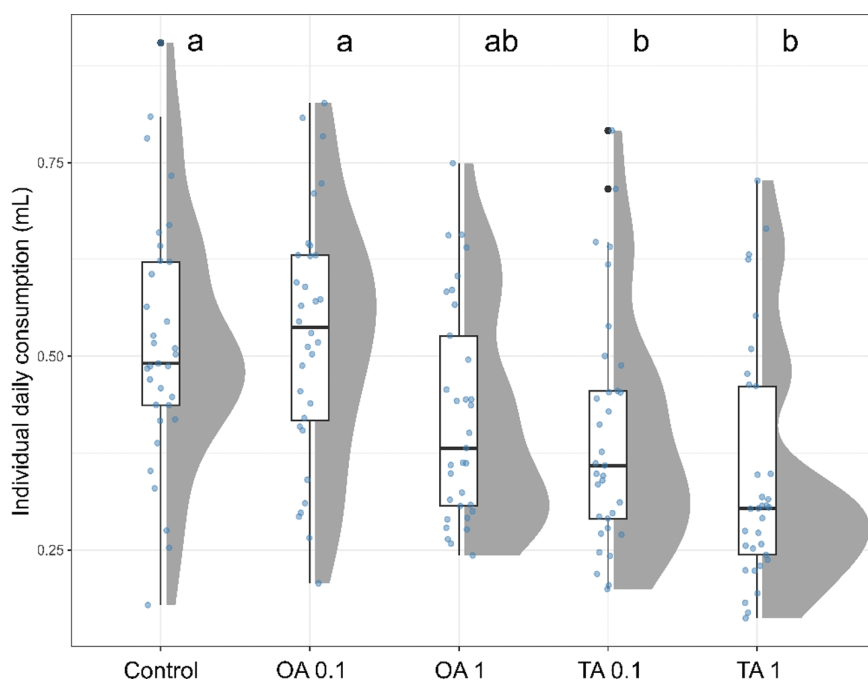


Figure 2. Mean individual daily consumption (ml) of the five treatment diets recorded for worker bumble bees caged by five in small flight cages (experiment 2). Different letters indicate significant differences among treatments. Control, $n=33$; OA 0.1, $n=32$; OA 1, $n=33$; TA 0.1, $n=33$; TA 1, $n=33$.

information). The subsequent post hoc test between pairwise contrasts highlighted no significant difference.

Bees fed OA 0.1 and OA 1 exhibited significantly less dynamic behavior than those fed Control (Control:OA 0.1, $t_{70}=2.837$, $p=0.046$; Control:OA 1, $t_{70}=2.735$, $p=0.059$) (Fig. 3b, Supporting information).

Feeding behavior (experiment 3)

The analysis of the fine feeding behavior of worker bumble bees showed no significant differences for any of the variables considered (duration of drinking, tasting and proboscis retrieving; frequency of tasting and proboscis retrieving; and total number of bouts, Supporting information), except for the frequency of drinking behavior recorded for bees fed TA 1, which was the lowest (TA 1: $t_{52}=-2.171$, $p=0.034$; Supporting information). Nevertheless, the subsequent pairwise post hoc test applied did not highlight any significant difference among treatments.

Discussion

Our findings confirm that the consumption of natural nectar concentrations of octopamine and tyramine have significant biological effects on various bumble bee behaviors which may be relevant to flower visitation. Though leaving open the question on whether ingested octopamine and tyramine can cross the blood-brain barrier, previous research on honey bees has demonstrated that oral intake increases brain levels of these compounds (Schulz and Robinson 2001), and that their consumption induces significant biological effects

on insect behavior (Barron et al. 2007, Agarwal et al. 2011, Arenas et al. 2020). However, most of such studies have used concentrations of biogenic amines hundreds or even thousands of times higher than those naturally found in floral nectar (Barberis et al. 2023c). A recent study conducted by Muth et al. (2023), for instance, investigated the effects of octopamine on the gustatory responsiveness of the species *Bombus impatiens* via conditioning of the proboscis extension reflex (PER) by means of a visual cue. The authors reported the first evidence of OA increasing gustatory responsiveness in harnessed bees at a relatively high concentration (52 mM), while such an effect is not retained when bees are fed solutions at a lower concentration (13 mM).

While Muth et al. (2022) emphasized the importance of testing combinations of biogenic amines on bee behavior – demonstrating that octopamine and tyramine administered together have no observable effect on bumblebees – our experimental approach was different. We based our choice on findings that report the mutually exclusive presence of octopamine and tyramine in the floral nectar of *Echium vulgare* (Barberis et al. 2023b, 2023c).

Our results suggest that octopamine at low concentrations increases food intake, which may indicate a preference for this compound. This result aligns with a study on adult blowflies (Long and Murdock 1983), where injection with an octopaminergic drug led to hyperphagia, demonstrating that octopaminergic receptors positively modulate feeding and drinking behavior in this species (Long and Murdock 1983). In addition, several studies have shown that both tyramine and octopamine enhance sucrose responsiveness in bees by increasing the perceived value of the reward,

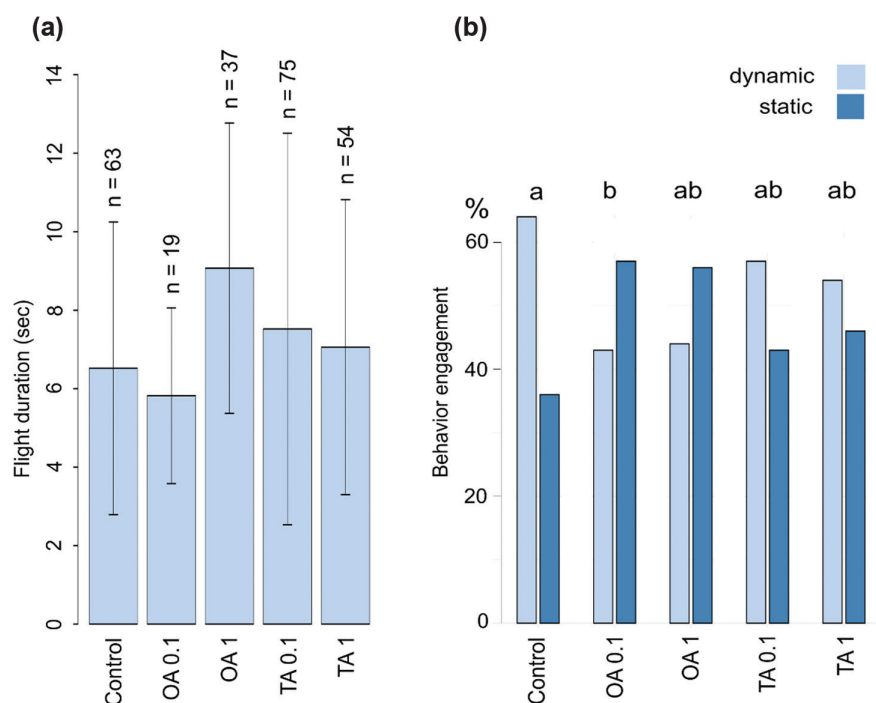


Figure 3. Mean \pm SD flight duration (a); n indicates the number of observations where a bee engaged in flight activity). The pairwise post hoc test following a GLMM did not highlight any significant difference among pairs of treatments. Percentage of dynamic versus static behavior (b) exhibited by bees fed different treatment diets and grouped by five in small flight cages. Different letters indicate statistically significant differences.

thereby promoting foraging behavior (Pankiw and Page 2003, McCabe et al. 2017). However, whether nectar concentrations of these compounds exert a similar effect remains unclear, as conflicting results emerged from several studies (Scheiner et al. 2002, Pankiw and Page 2003, Muth et al. 2022).

In our study, the general trend towards a preference for octopamine over tyramine became more pronounced when observed over a longer period in individually caged bees, which were confined into a Nicot cage, therefore unable to fly or engage in extensive walking (as in experiment 1). These bees consumed more solution when fed a diet enriched with the lowest concentration of octopamine (OA 0.1). Although this trend was not statistically significant in experiment 2, it was also observed in bees grouped in small flight cages, where they could engage in short flight and walking. The results from experiment 2 showed that tyramine-enriched solutions were consumed less than the control solution, rather than indicating a preference for octopamine-enriched diets. Since experiment 2 was conducted in a slightly more natural setting for social insects, with bumble bees housed in larger cages in groups of five instead of individually, this may suggest that the significantly greater consumption of octopamine-enriched solution at lower concentrations in experiment 1 could have been a stress-induced response. Additionally, bees fed tyramine did not show any significant difference in overall dynamism compared to those fed control diets. These findings suggest that bees may find tyramine-enriched solutions less appealing than octopamine-enriched solutions, or that

tyramine is less phagostimulant. Yet, in experiment 3, tyramine did not significantly increase the frequency of interruptions in drinking behavior compared to the other treatments.

No significant effect on consumption was detected as an immediate response to the treatment diet during short-term exposure (experiment 3). Insect pollinators can assess the quality of nectar and pollen using their peripheral organs. Antennae, mouthparts and legs are covered with different types of sensilla housing gustatory receptor neurons (GRNs), which allow insects to taste many chemical compounds, including some secondary metabolites (Lim et al. 2019, Ruedenauer et al. 2019, Bestea et al. 2021, Rossoni et al. 2025). However, not many studies have thoroughly examined the gustatory system of bees, particularly their ability to detect secondary metabolites in nectar (reviewed by de Brito Sanchez 2011, Bestea et al. 2021), and whether pollinators can specifically taste biogenic amines is currently unknown. That being said, since in experiment 3 all treatment diets were based on 50% w/v sucrose solutions, the high sugar concentration may have masked any potential preference linked to differences in palatability. Moreover, the high sucrose concentration used may have induced a ceiling effect, limiting the ability to detect variations in perceived value of the reward that could have been induced by tyramine and octopamine, as suggested by Pankiw and Page (2003) and McCabe et al. (2017). It is worth noting that a preference for laced over plain artificial nectar does not necessarily require the bees to be able to taste them. For instance, honey bees and bumble bees have been shown to prefer sucrose solutions

containing neonicotinoid pesticides, despite these chemicals not eliciting spiking responses from GRNs in the mouthparts (Kessler et al. 2015). Both pre- and post-ingestive processes may indeed account for food preference or even avoidance learning for certain nectar toxins. Therefore, it is possible that the increased food consumption observed in bees fed 0.1 mM octopamine solution in experiment 1 may be due to post-ingestive effects, such as phagostimulation.

In our study, bumble bees could not engage in prolonged flights within the observation cages but instead exhibited short flight hops. Consequently, our data may not fully reflect the actual duration of sustained flight that the subjects might have engaged in under natural conditions. Rather, they highlight the extent to which the bees were motivated to fly. Previous studies have shown that octopamine and tyramine differentially affect flight behavior in honeybees when injected into the thorax. Specifically, octopamine tends to enhance flight, while tyramine generally reduces it (Roeder 2005, Fussnecker et al. 2006). Our findings are only partially consistent with these results: octopamine, at a lower natural concentration, decreased the bees' motivation to fly. In contrast, bees fed a treatment diet enriched with a higher octopamine concentration – while not exhibiting a high frequency of flight – showed, on average, longer flight durations. However, at natural nectar concentrations, tyramine appeared to have no significant effect on either the frequency or duration of flight compared to the control.

Our study was conducted in a laboratory setting. While the use of captive animals presents limitations, it generally facilitates the identification of behavioural effects induced by different artificial nectars (Barberis et al. 2025a). Despite controlled settings simplifying the system by excluding a number of confounding variables, they may not always provide an entirely realistic representation of natural behavior. For instance, harnessed bees can behave differently from free-moving individuals (Ayestaran et al. 2010). Even though we recognize that experiments with caged bees should be preferably followed by essays on freely flying bees, our results can be used to infer what may occur in more realistic scenarios. Plant–pollinator co-evolution, which has lasted for more than 100 million years, has made both plants and pollinators reciprocally dependent upon one another (Mitchell et al. 2009). Particularly, plants have evolved flowers that enhance their attractiveness and fitness, making themselves more visible and memorable with shapes, colors and volatile organic compounds (VOCs), and more attractive with complex nectars (Chittka and Menzel 1992, Raguso 2004, Moyroud and Glover 2017), so that they are easily recognizable and memorable even in the framework of complex communities. Nectar composition has been shaped by pollinators and its high complexity due to secondary metabolites appears to play a crucial role in building pollinator loyalty (Nepi et al. 2018, Mustard 2020). Therefore, our results suggest that also the presence of biogenic amines in nectar may have the potential to influence pollinator foraging behavior in nature, a hypothesis that needs to be validated in mesocosms and field experiments.

Conclusion and future perspectives

Based on the concentrations of tyramine and octopamine reported in previous studies (Muth et al. 2022, Barberis et al. 2023b, 2023c) our treatment diets at 0.1 mM seem to more closely reflect the typical levels of these compounds in the natural nectar of various species. At this concentration, octopamine in floral nectar may enhance insect retention, either by stimulating feeding behavior or reducing their motivation to fly away. It is worth noting that the neurobiological mechanisms underlying foraging behavior are not only driven by external cues or learned associations but also by the insect's internal state. This state is regulated by neuromodulators such as dopamine and octopamine, as well as neuropeptides like FMRFamide-related peptides, which influence hedonic components of motivation, including “wanting” and “liking” that shape the feeding behavior (Huang et al. 2022, Bastea et al. 2022, Da Silva et al. 2025). These hedonic processes may also be linked to primitive emotional systems, which are increasingly recognized in insects and thought to play a role in adaptive decision-making (Perry and Barron 2013, Baracchi et al. 2017b). Dopamine, for example, plays a role in aligning foraging effort with both individual nutritional status and colony-level needs (Huang et al. 2022), while octopamine acts as a value-encoding system in appetitive learning (Giurfa 2006, Perry and Barron 2013). Although we did not manipulate these pathways directly, their likely involvement suggests that nectar chemistry may influence pollinator behaviour by engaging core motivational and affective systems. If these effects were confirmed, they would indicate that the presence of these compounds in floral nectar can influence plant reproductive success in different ways, depending on species-specific traits like factors such as the plant's breeding system. Therefore, different model plant systems should be studied individually to fully understand how these compounds affect plant reproductive fitness. Ultimately, what our study indicates is that insect pollinators that interact with the nectar landscape consume pharmacologically active concentrations of exogenous neurotransmitters, and this may influence behaviors potentially crucial to flower visits.

Acknowledgements – We want to thank Boštjan Surina for hosting Marta Barberis at the Natural History Museum Rijeka allowing her to perform the video analyses using the event logging software Mangold Interact (Mangold International GmbH, Arnstorf, Germany). Open access publishing facilitated by Università degli Studi di Bologna, as part of the Wiley – CRUI-CARE agreement.

Funding – This study was partially supported by the project BeeNet (Italian National Fund under FEASR 2014-2020) from the Italian Ministry of Agriculture, Food Sovereignty and Forestry (MASAF). MN and DC were partially supported by the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree 410 n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU. Project code CN_00000033, CUP B63C22000650007, Project title “National Biodiversity Future

Center - NBFC Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research. MG was partially supported by the Italian National Recovery and Resilience Plan (NRRP), Project title "National Biodiversity Future Center -NBFC". CUP J33C22001190001. MB was supported by both a PhD scholarship from the University of Bologna and a Marco Polo Grant during her period abroad.

Author contributions

Marta Barberis and **Daniele Calabrese** contributed equally to this publication. **Marta Barberis**: Conceptualization (equal); Formal analysis (lead); Methodology (equal); Writing – original draft (lead). **Daniele Calabrese**: Conceptualization (equal); Methodology (equal); Writing – original draft (lead). **David Baracchi**: Writing – review and editing (equal). **Laura Bortolotti**: Conceptualization (equal); Writing – review and editing (equal). **Flavia Di Cesare**: Data curation (equal); Investigation (equal); Methodology (equal); Writing – review and editing (equal). **Rosa Ranalli**: Data curation (equal); Investigation (equal); Methodology (equal); Writing – review and editing (equal). **Laura Zavatta**: Data curation (equal); Investigation (equal); Methodology (equal); Writing – review and editing (equal). **Massimo Nepi**: Conceptualization (equal); Writing – review and editing (equal). **Marta Galloni**: Conceptualization (equal); Writing – review and editing (equal). **Gherardo Bogo**: Conceptualization (equal); Data curation (equal); Methodology (equal); Supervision (lead); Writing – review and editing (equal).

Data availability statement

Data are available from Zenodo: <https://doi.org/10.5281/zenodo.14749336> (Barberis et al. 2025b).

Supporting information

The Supporting information associated with this article is available with the online version.

References

Agarwal, M., Giannoni Guzmán, M., Morales-Matos, C., Del Valle Díaz, R. A., Abramson, C. I. and Giray, T. 2011. Dopamine and octopamine influence avoidance learning of honey bees in a place preference assay. – *PLoS One* 6: e25371.

Arenas, A., Lajad, R., Peng, T., Grüter, C. and Farina, W. 2020. Correlation between octopaminergic signaling and foraging task specialization in honeybees. – *Genes Brain Behav.* 20: e12718.

Ayestaran, A., Giurfa, M. and de Brito Sanchez, M. G. 2010. Toxic but drunk: gustatory aversive compounds induce post-ingestional malaise in harnessed honeybees. – *PLoS One* 5: e15000.

Baker, H. G. and Baker, I. 1986. The occurrence and significance of amino acids in floral nectars. – *Plant Syst. Evol.* 151: 175–186.

Baracchi, D., Cabirol, A., Devaud, J.-M., Haase, A., d’Ettorre, P. and Giurfa, M. 2020. Pheromone components affect motivation and induce persistent modulation of associative learning and memory in honey bees. – *Commun. Biol.* 3: 447.

Baracchi, D., Marples, A., Jenkins, A. J., Leitch, A. R. and Chittka, L. 2017a. Nicotine in floral nectar pharmacologically influences bumblebee learning of floral features. – *Sci. Rep.* 7: 1951.

Baracchi, D., Lihoreau, M. and Giurfa, M. 2017b. Do insects have emotions? Some insights from bumble bees. – *Front. Behav. Neurosci.* 11: 157.

Barberis, M., Calabrese, D., Galloni, M. and Nepi, M. 2023a. Secondary metabolites in nectar-mediated plant–pollinator interactions. – *Plants* 12: 550.

Barberis, M., Bogo, G., Bortolotti, L., Guarnieri, M., Nepi, M., Felicioli, A. and Galloni, M. 2023b. Nectar tyramine decreases the duration of bumblebee visits on flowers. – *Arthropod Plant Interact.* 17: 563–569.

Barberis, M., Bogo, G., Bortolotti, L., Flaminio, S., Giordano, E., Nepi, M. and Galloni, M. 2023c. Floral nectar and insect flower handling time change over the flowering season: results from an exploratory study. – *Acta Oecol.* 120: 103937.

Barberis, M., Nepi, M. and Galloni, M. 2024. Floral nectar: fifty years of new ecological perspectives beyond pollinator reward. – *Perspect. Plant Ecol. Evol. Syst.* 62: 125764.

Barberis, M., Iovane, M. and Pozo, M. 2025a. Pollination unveiled: exploring the floral rewards. – In: Cilia, G., Ranalli, R., Zavatta, L. and Flaminio, S. (eds), *Hidden and wild: an integrated study of European wild bees*. Springer.

Barberis, M., Calabrese, D., Baracchi, D., Bortolotti, L., Di Cesare, F., Ranalli, R., Zavatta, L., Nepi, M., Galloni, M. and Bogo, B. 2025b. Data from: Nectar concentrations of biogenic amines affect bumble bee behavior in a dose-dependent manner. – Zenodo, <https://doi.org/10.5281/zenodo.14749336>.

Barron, A. B., Maleszka, R., Vander Meer, R. K. and Robinson, G. E. 2007. Octopamine modulates honey bee dance behavior. – *Proc. Natl Acad. Sci. USA* 104: 1703–1707.

Bestea, L., Réjaud, A., Sandoz, J.-C., Carcaud, J., Giurfa, M. and de Brito Sanchez, M. G. 2021. Peripheral taste detection in honey bees: what do taste receptors respond to? – *Eur. J. Neurosci.* 54: 4417–4444.

Blenau, W. and Baumann, A. 2001. Molecular and pharmacological properties of insect biogenic amine receptors: lessons from *Drosophila melanogaster* and *Apis mellifera*. – *Arch. Insect Biochem. Physiol.* 48: 13–38.

Bogo, G., Bortolotti, L., Sagona, S., Felicioli, A., Galloni, M., Barberis, M. and Nepi, M. 2019. Effects of non-protein amino acids in nectar on bee survival and behavior. – *J. Chem. Ecol.* 45: 278–285.

Bogo, G., Fisogni, A., Rabassa-Juventeny, J., Bortolotti, L., Nepi, M., Guarnieri, M., Conte, L. and Galloni, M. 2021. Nectar chemistry is not only a plant's affair: floral visitors affect nectar sugar and amino acid composition. – *Oikos* 130: 1180–1192.

Bogo, G., Fisogni, A., Barberis, M., Ranalli, R., Zavatta, L., Bortolotti, L., Felicioli, A., Massol, F., Nepi, M., Rossi, M., Sagona, S. and Galloni, M. 2024. Proline and β -alanine influence bumblebee nectar consumption without affecting survival. – *Apidologie* 55: 46.

Carlesso, D., Smargiassi, S., Pasquini, E., Bertelli, G. and Baracchi, D. 2021. Nectar non-protein amino acids (NPAAs) do not change nectar palatability but enhance learning and memory in honey bees. – *Sci. Rep.* 11: 11721.

Chittka, L. and Menzel, R. 1992. The evolutionary adaptation of flower colours and the insect pollinators' colour vision. – *J. Compar. Physiol. A* 171: 171–181.

da Silva, R. C., Baracchi, D., Ricciardi, G., Giurfa, M. and de Brito Sanchez, M. G. 2025. Seasonal and nutritional modulation of

- honeybee olfactory learning by the short neuropeptide F. – *Proc. R. Soc. B* 292: 20250655.
- de Brito Sanchez, M. G. 2011. Taste perception in honey bees. – *Chem. Senses* 36: 675–692.
- Farooqui, T. 2012. Review of octopamine in insect nervous systems. – *Open Access Insect Physiol.* 4: 1–17.
- Farooqui, T., Robinson, K., Vaessin, H. and Smith, B. H. 2003. Modulation of early olfactory processing by an octopaminergic reinforcement pathway in the honey bee. – *J. Neurosci.* 23: 5370–5380.
- Finetti, L., Roeder, T., Calò, G. and Bernacchia, G. 2021. The insect type 1 receptors: from structure to behavior. – *Insects* 12: 315.
- Fussnecker, B. L., Smith, B. H. and Mustard, J. A. 2006. Octopamine and tyramine influence the behavioral profile of locomotor activity in the honey bee (*Apis mellifera*). – *J. Insect Physiol.* 52: 1083–1092.
- Giurfa, M. 2006. The instructive function of biogenic amines in insect associative learning. – *Curr. Biol.* 16: R892–R895.
- Hammer, M. 1993. An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honey bees. – *Nature* 366: 59–63.
- Hammer, M. and Menzel, R. 1998. Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honey bees. – *Learn. Mem.* 5: 146–156
- Huang, J., Zhang, Z., Feng, W., Zhao, Y., Aldanondo, A., de Brito Sanchez, M. G., Paoli, M., Rolland, A., Li, Z., Nie, H., Lin, Y., Zhang, S., Giurfa, M. and Su, S. 2022. Food wanting is mediated by transient activation of dopaminergic signaling in the honey bee brain. – *Science* 376: 508–512.
- Kassambara, A., Kosinski, M. and Biecek, P. 2021. *Survminer*: drawing survival curves using *ppplot2*. – R package ver. 0.4.9, <https://CRAN.R-project.org/package=survminer>.
- Kessler, S., Tiedeken, E. J., Simcock, K. L., Derveau, S., Mitchell, J., Softley, S., Stout, J. C. and Wright, G. A. 2015. Bees prefer foods containing neonicotinoid pesticides. – *Nature* 521: 74–76
- Kononenko, N. L., Wolfenberger, H. and Pflüger, H.-J. 2009. Tyramine as an independent transmitter and a precursor of octopamine in the locust central nervous system: an immunocytochemical study. – *J. Comp. Neurol.* 512: 433–452.
- Lim, S., Jung, J., Yunusbaev, U., Ilyasov, R. and Kwon, H. W. 2019. Characterization and its implication of a novel taste receptor detecting nutrients in the honey bee, *Apis mellifera*. – *Sci. Rep.* 9: 11620.
- Linn, M., Glaser, S. M., Peng, T. and Grüter, C. 2020. Octopamine and dopamine mediate waggle dance following and information use in honeybees. – *Proc. R. Soc. B* 287: 20201950.
- Long, T. F. and Murdock, L. L. 1983. Stimulation of blowfly feeding behavior by octopaminergic drugs. – *Proc. Natl. Acad. Sci. USA* 80: 4159–4163.
- Ma, C., Kessler, S., Simpson, A. and Wright, G. A. 2016. A novel behavioral assay to investigate gustatory responses of individual, freely-moving bumble bees (*Bombus terrestris*). – *J. Vis. Exp.* 113: e54233.
- McCabe, S. I., Ferro, M. W. B., Farina, W. M. and Hrcir, M. 2017. Dose- and time-dependent effects of oral octopamine treatments on the sucrose responsiveness in stingless bees (*Melipona scutellaris*). – *Apidologie* 48: 1–7.
- Mitchell, R. J., Irwin, R. E., Flanagan, R. J. and Karron, J. D. 2009. Ecology and evolution of plant–pollinator interactions. – *Ann. Bot.* 103: 1355–1363.
- Moyroud, E. and Glover, B. J. 2017. The physics of pollination attraction. – *New Phytol.* 216: 350–354.
- Mustard, J. A. 2020. Neuroactive nectar: compounds in nectar that interact with neurons. – *Arthropod Plant Interact.* 14: 151–159.
- Muth, F., Philbin, C. S., Jeffrey, C. S. and Leonard, A. S. 2022. Discovery of octopamine and tyramine in nectar and their effects on bumblebee behaviour. – *iScience* 25: 104765.
- Muth, F., Breslow, E. and Leonard, A. S. 2023. Octopamine affects gustatory responsiveness and may enhance learning in bumble bees. – *Apidologie* 54: 9.
- Nepi, M. 2014. Beyond nectar sweetness: the hidden ecological role of non-protein amino acids in nectar. – *J. Ecol.* 102: 108–115.
- Nepi, M., Grasso, D. A. and Mancuso, S. 2018. Nectar in plant–insect mutualistic relationships: from food reward to partner manipulation. – *Front. Plant Sci.* 9: 1063.
- Orchard, I. 1982. Octopamine in insects: neurotransmitter, neurohormone, and neuromodulator. – *Can. J. Zool.* 60: 659–669.
- Palmer-Young, E. C., Farrell, I. W., Adler, L. S., Milano, N. J., Egan, P. A., Junker, R. R., Irwin, R. E. and Stevenson, P. C. 2019. Chemistry of floral rewards: intra- and inter-specific variability of nectar and pollen secondary metabolites across taxa. – *Ecol. Monogr.* 89: e01335.
- Pankiw, T. and Page, R. E. Jr. 2003. Effect of pheromones, hormones, and handling on sucrose response thresholds of honey bees (*Apis mellifera* L.). – *J. Comp. Physiol. A* 189: 675–684.
- Peng, T., Schroeder, M. and Grüter, C. 2020. Octopamine increases individual and collective foraging in a Neotropical stingless bee. – *Biol. Lett.* 16: 20200238.
- Perry, C. J. and Barron, A. B. 2013. Neural mechanisms of reward in insects. – *Annu. Rev. Entomol.* 58: 543–562.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., EISPACk authors, Heisterkamp, S., Van Willigen, B., Ranke, J. and R Core Team 2022. Linear and non-linear mixed effects models. – <https://CRAN.R-project.org/package=nlme>.
- Raguso, R. A. 2004. Why are some floral nectars scented? – *Ecology* 85: 1486–1494.
- Rizopoulos, D. 2022. *GLMMadaptive*: generalized linear mixed models using adaptive gaussian quadrature. – R package ver. 0.8-5, <https://CRAN.R-project.org/package=GLMMadaptive>.
- Roeder, T. 2000. Octopamine in invertebrates. – *Prog. Neurobiol.* 59: 533–561.
- Roeder, T. 2005. Tyramine and octopamine: ruling behavior and metabolism. – *Annu. Rev. Entomol.* 50: 447–477.
- Rossoni, S., Parkinson, R. H., Niven, J. E. and Nicholls, E. 2025. Gustatory sensitivity to amino acids in bumblebee mouthparts. – *R. Soc. Open Sci.* 12: 250465.
- Scheiner, R., Plückhahn, S., Öney, B., Blenau, W. and Erber, J. 2002. Behavioural pharmacology of octopamine, tyramine and dopamine in honeybees. – *Behav. Brain Res.* 136: 545–553.
- Scheiner, R., Baumann, A. and Blenau, W. 2006. Aminergic control and modulation of honey bee behaviour. – *Curr. Neuropharmacol.* 4: 259–276.
- Schulz, D. J. and Robinson, G. E. 2001. Octopamine influences division of labor in honey bee colonies. – *J. Comp. Physiol. A* 187: 53–61.
- Stevenson, P. C., Nicolson, S. W. and Wright, G. A. 2017. Plant secondary metabolites in nectar: impacts on pollinators and ecological functions. – *Funct. Ecol.* 31: 65–75.
- Thomson, J. D., Draguleasa, M. A. and Tan, M. G. 2015. Flowers with caffeinated nectar receive more pollination. – *Arthropod Plant Interact.* 9: 1–7.
- Wright, G. A., Baker, D. D., Palmer, M. J., Stabler, D., Mustard, J. A., Power, E. F., Borland, A. M. and Stevenson, P. C. 2013. Caffeine in floral nectar enhances a pollinator's memory of reward. – *Science* 339: 1202–1204.