

SHORT COMMUNICATION

Context dependency of in-flight responses by *Manduca sexta* moths to ambient differences in relative humidity

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ABSTRACT

The use of sensory information to control behavior usually involves the integration of sensory input from different modalities. This integration is affected by behavioral states and experience, and it is also sensitive to the spatiotemporal patterns of stimulation and other general contextual cues. Following the finding that hawkmoths can use relative humidity (RH) as a proxy for nectar content during close-range foraging, we evaluate here whether RH could be used during locomotive flight under two simulated contexts in a wind tunnel: (1) dispersion and (2) search phase of the foraging behavior. Flying moths showed a bias towards air with a higher RH in a context devoid of foraging stimuli, but the addition of visual and olfactory floral stimuli elicited foraging responses that overrode the behavioral effects of RH. We discuss the results in relation to the putative adaptive value of the context-dependent use of sensory information.

KEY WORDS: Sensory ecology, Behavior, Lepidoptera, Multimodal, Environment

INTRODUCTION

Animals use different types of sensory information, including visual and olfactory cues, to identify flowers as sources of nectar, pollen and other resources (reviewed by Raguso, 2004; Chittka and Raine, 2006). Recent studies have expanded the list of sensory channels used by pollinators during flower foraging. For example, bats can use acoustic signatures from flower petals (or even leaves) to locate floral nectar (von Helversen and von Helversen, 2003; Simon et al., 2011), and bumblebees can use static electric charge as reliable information about the profitability of individual flowers (Clarke et al., 2013). Floral temperature is important both to carrion flies visiting brood site-deceptive flowers (Angioy et al., 2004) and to bumblebees in more typical nectar-based foraging behavior (Whitney et al., 2008). Finally, relative humidity gradients produced by floral nectar provide hawkmoths with honest index cues for the presence of nectar (von Arx et al., 2012).

A question common to these studies is: to what extent do pollinators integrate information from different sensory channels as multi-modal stimuli (Leonard et al., 2011)? Nectar-feeding bats in the family Phyllostomidae can use either olfactory or acoustic channels to detect and locate flowers, but show a preference for the

combination thereof (Gonzalez-Terrazas et al., 2016). Multi-modal interactions have been well studied in tobacco hornworm moths (*Manduca sexta*; Sphingidae), which are large, long-tongued nectar-feeding insects that pollinate guilds of night-blooming flowers. These hawkmoths primarily use olfactory and visual cues in nectar foraging, with complex spatiotemporal interactions between these inputs governing specific behavioral outputs at different distances from the flower (Raguso and Willis, 2002; Goyret et al., 2007). Recent studies have explored how non-specific plant metabolites such as CO₂ impact the nectar-foraging behavior of *M. sexta*. In a wind tunnel, CO₂ influences the upwind orientation flight of *M. sexta* moths towards a surrogate flower in the same way as floral odors. Nevertheless, once moths approach the flower, CO₂ has no effect on the probing responses, indicating a context-dependent effect of CO₂ on foraging behavior (Goyret et al., 2008). In additional dual-choice experiments, male moths showed a strong preference for flowers emitting a floral scent and CO₂ over flowers emitting only scent, but female moths showed similar biases only when CO₂ was presented in an oviposition context, along with host plant (tomato) leaf volatiles (see Adler and Bronstein, 2004). Thus, the effects of specific and non-specific foraging cues depend on the scale and context under which they are perceived.

Nevertheless, the context-dependent roles of non-specific cues such as floral CO₂ are seldom investigated at spatial scales beyond that of the flower, and remain poorly understood for most plant–pollinator relationships. Relative humidity (RH) provides an excellent example, as it is likely to impact pollinator behavior very differently at a landscape scale, where RH might indicate appropriate habitat (affecting orientation/dispersal behaviors), than at the scale of a flower patch, where RH might indicate profitability if tied to plant quality or nectar availability (affecting feeding-specific behaviors). Our recent studies with hawkmoths support the suggestion that RH could provide different kinds of information under different contexts. In a foraging context, Von Arx et al. (2012) explored differences in RH as close-range floral cues for the hawkmoth *Hyles lineata*. The nectar within long-tubed, night-blooming flowers of *Oenothera cespitosa* (Onagraceae) produces a local RH gradient (5–8% above ambient) that is present when the flower opens, and moths in flight cage bioassays prefer to approach and probe at artificial flowers with these slightly elevated RH levels over neighboring flowers with ambient RH values. When differences in RH are evaluated at the habitat scale, there are consequences for hawkmoth survival as well as foraging behavior. Contreras et al. (2013) showed that adult *M. sexta* moths alter their nectar-feeding behavior when they are housed in experimental growth chambers at different ambient RH levels. Moths preferred more dilute nectar at low ambient RH, presumably to meet their osmotic demands, with no concomitant change in energy acquired from the nectar. Moreover, mean survivorship of *M. sexta* doubled when ambient RH increased from 20% to 80% (Contreras et al., 2013), supporting the suggestion that adult hawkmoths should

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disperse from seasonally dry habitats to more humid habitats when such behavior is possible (Janzen, 1984; Powell and Brown, 1990).

Despite the progress indicated by these studies, we lack a comprehensive understanding of how hawkmoths and other flower-visiting animals use RH at different spatial scales and in the presence of different floral stimuli. In parallel with previous experiments decoupling floral scent, visual cues and CO₂ (Goyret et al., 2008), in this study we used wind tunnel assays to observe in-flight orientation responses of *M. sexta* moths to small differences in RH (5–8%), with and without floral stimuli (visual and olfactory cues).

In the first experiment, we tested whether small RH differences could affect upwind flight by moths in a wind tunnel. In the second experiment, we tested whether a foraging sensory context could affect how moths responded to the conditions of the first experiment by adding floral visual and olfactory stimuli to the same experimental arena. Our findings provide another instance of context-dependent responses of animals to sensory information, addressing a deeper conceptual gap in our understanding of nuanced, flexible foraging behavior.

MATERIALS AND METHODS

Insects

We used naive adult *M. sexta* (Linnaeus 1763) moths from our laboratory colony at Cornell University (Ithaca, NY, USA). Larvae were reared on artificial diet (Goyret and Yuan, 2015) and were kept in a growth chamber at 24±1°C, 60±5% RH, on a 16 h:8 h light:dark cycle. Pupae were separated by sex and were transferred to mesh cages in two different incubators held under the same conditions, where they remained until 2–4 days post-eclosion. All moths used for assays were unmated, flower naive and were starved for 2–4 days before being tested.

Flight tunnel

We measured the responses of *M. sexta* moths under different conditions by using binary-choice tests in a laminar flow flight tunnel. The flight tunnel was 2 m length×1 m width×1 m height with a fan installed at the upwind end to create airflow through the tunnel at 1.2 m s⁻¹. The upwind end was divided down the midline from floor to ceiling by a 60 cm long acrylic septum. Screens were placed at both ends of the tunnel to provide laminar airflow, and air was drawn out through a fume hood at the downwind end of the tunnel. Light was provided from above, with the light intensity (illuminance) reduced to 2 lx at floor level to simulate the twilight conditions at which crepuscular hawkmoths become active (Johnsen et al., 2006).

A commercial humidifier was placed between the fan and the upwind screen to elevate the RH in one of the two halves of the tunnel defined by the acrylic septum (hereafter termed the humid and ambient sides), and cardboard baffles were placed in the other

half of this space to balance air flow, which we confirmed using a hot-wire anemometer (425 thermal anemometer, Testo Inc., Sparta Township, NJ, USA). The side on which the humidifier was placed was changed each day of the flight experiments in a pseudorandom fashion.

We measured RH at 30 different positions in the flight tunnel (Fig. 1) using an HMI41 indicator with an HMP46 humidity probe (Vaisala Inc., Helsinki, Finland) to visualize the RH profile 60 cm above the tunnel floor, the height at which moths were released. A heat map was generated to visualize the RH differences in the wind tunnel [QGIS version 2.16; Thin Plate Spline (Global) algorithm] using mean RH values at each point ($n=4$), in which the size of each point corresponds to the standard error of the mean at that position. This heat map is representative of the conditions in the flight tunnel during experiments 1 and 2 (see below). Temperature and RH were measured at standard positions 'A' (ambient) and 'H' (humid; see Fig. 1) before each replicate, and were used as internal standards in both experiments. The average difference in RH at these positions was 5.7±0.3%.

The ambient RH ranged from 14.5% to 47.9%. The average RH was 44.1±4% at position H and 32.7±1.9% at position A. The average RH at the release point was 38.3±3.2%. Thus, we were able to experimentally manipulate RH on one side of the flight tunnel, such that flying test moths were presented with two relatively non-turbulent air streams that differed in RH by 5–12%. A comparable scale of RH differences (5–13% RH) is observed between xeric slopes in the Santa Rita Experimental Range (Pima County, AZ, USA) and adjoining woodlands, albeit at a 2 km distance (G.D., unpublished data), where previous field studies of *M. sexta* have been performed (Alarcón et al., 2008; Bronstein et al., 2009). Thus, our experimental manipulation of RH is ecologically relevant to the RH differences experienced by flying *M. sexta* moths in their peak activity period, during the summer monsoon season of the Sonoran Desert (see Contreras et al., 2013).

Treatments

In experiment 1, as individual moths progressed in their upwind flight in the wind tunnel, they were exposed to airstreams of different RH in the absence of floral stimuli. In experiment 2, we added artificial flowers (as designed by Goyret et al., 2007) to both sides of the flight tunnel, otherwise using the same setup as in experiment 1 to present moths with a lateral RH difference in moving air. Cotton swabs placed within each artificial flower were scented with two drops of bergamot oil. Bergamot oil is chemically similar to the floral odors released by hawkmoth-pollinated flowers and is a reliable feeding attractant for *M. sexta* (Goyret and Raguso, 2006). Additional assays were run in the flight tunnel under ambient conditions (no humidity or floral stimuli present) to control for any possible side-bias effect.

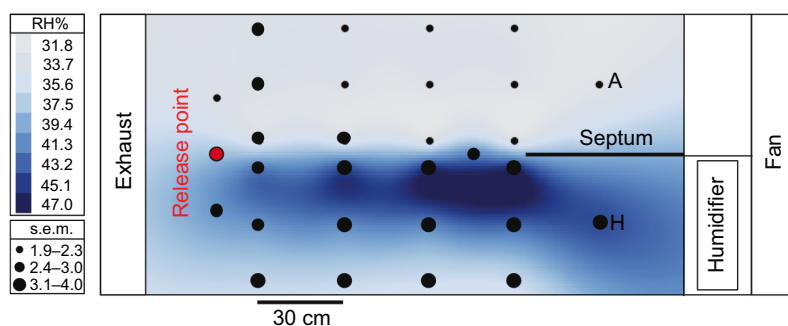


Fig. 1. Flight tunnel setup with relative humidity profile. The relative humidity (RH) was recorded at 30 points (black dots) in the flight tunnel ($n=3$) and average values were calculated. Colors depict the average percentage RH above the average ambient RH (31.8%), where humid air is blue and ambient air is gray. Dot sizes represent s.e.m. of the measurements at each location.

Assays

Moths were tested in cohorts (1–12 moths per day) so that all assays could be performed within the first 90 min of the dark period (scotophase). Moths were assayed individually and only once in the flight tunnel. The temperature and RH on each side of the acrylic septum were recorded before each flight. Moths were transferred individually to cylindrical aluminium screen release cages and transported to the flight tunnel room 2 h prior to scotophase. Each moth was placed at the release point (Fig. 1) in the flight tunnel and its behavior was observed for up to 5 min. Moths that did not take flight, or that flew for less than 30 s, were not considered for analysis. A choice was scored if a moth crossed the boundary defined by the edge of the septum (Fig. 1) and remained there for at least 2 s. In experiment 1, we scored subsequent choices with the same criterion, but only if a moth flew and remained downwind of the choice areas for more than 5 s before reaching one of them again. In experiment 2, only the first side choice was scored for each moth tested, and flower visitation (probing) was recorded.

Statistical analysis

Binomial tests were used to analyze side preference for both experiments. Residence time (s) in each choice area was also recorded for both experiments, and was analyzed using Wilcoxon signed-rank tests (SPSS 24; IBM Corporation) because both sets of data were not normally distributed.

RESULTS AND DISCUSSION

In experiment 1, moths showed a significant bias toward the more humid side of the flight tunnel for their first ($n=60$; binomial test, $P=0.0008$) and second choice ($n=43$; $P=0.0042$), but no significant tendency was detected for the third ($n=26$; $P=0.0792$) or fourth choices ($n=15$; $P=0.1964$). Overall, arrivals at the downwind end were significantly biased toward the humid side ($n=144$; $P<0.0001$; Fig. 2). Moths did not display a significant side bias in control assays ($n=20$; binomial test, $P=0.1602$; Fig. 3). We found no sex differences in either experiment 1 ($G_h=0.629$; $P=0.4275$) or the control ($G_h=0.641$; $P=0.4231$; data not shown). Moths spent significantly more time on the humid side (17.5 ± 2.6 s) than on the ambient side (2.9 ± 0.5 s) of the tunnel ($n=60$; Wilcoxon signed-rank test; $Z=-5.359$; $P<0.0001$).

In experiment 2, when visual and olfactory floral stimuli were present on both sides of the flight tunnel, moths did not display a

significant bias for the more humid side ($n=43$; binomial test, $P=0.1$; Fig. 3; sex was not recorded in this experiment). The average difference in RH at locations H and A was $8.2\pm 0.6\%$. When floral stimuli were present, residence times in each side of the tunnel did not differ significantly ($n=43$; humid side: 107.6 ± 15.6 s; ambient side: 96.5 ± 14.3 s; Wilcoxon signed-rank test; $Z=-368$; $P=0.713$). Of the 43 moths that reached the choice point, 23 visited at least one artificial flower. Moths showed a non-significant side bias for their first flower probing (humid side: $n=15$, ambient side: $n=8$; $P=0.0584$) and for the overall probes (humid side: $n=15$, ambient: $n=14$; $P=0.1445$).

Hawkmoths often live in seasonal environments that undergo extreme shifts in abiotic (and associated biotic) conditions (Janzen, 1987a; Contreras et al., 2013). The tropical dry forest of Santa Rosa National Park (Guanacaste Province, Costa Rica) has a 6 month rain-free dry season (Janzen, 1987a), just as the Sonoran Desert straddling the USA–Mexico border is subject to large seasonal changes in temperature, RH and precipitation (Unland et al., 1996; Davidowitz, 2002); these sites contain two of the best-studied hawkmoth faunas in the world. During the dry season, hawkmoths commonly located in lowland areas also can be found in more temperate mountain passes, and there is evidence of cyclic migration or dispersal between dry and wet environments (Janzen, 1984, 1987b; Powell and Brown, 1990). Hawkmoth movements could be mediated through the use of regional humidity gradients, among other mechanisms. Experiment 1 was designed to test whether hawkmoths could detect small differences in RH and utilize these differences as an in-flight orientation cue in a wind tunnel.

Small RH differences had a strong effect on the flight and overall residence times of moths in the wind tunnel, which suggests that even modest RH gradients can be used effectively as orientation cues. The question remains whether this is the result of avoiding drier air or preferring humid air. It is challenging to experimentally replicate the dynamic, open landscapes that hawkmoths regularly traverse (Suzán et al., 1994) without compromising important aspects of orientation, including flight speed (Stevenson et al., 1995). Despite these caveats, our results indicate that RH differences may be detected in-flight and, in the confines of a relatively small trajectory of a wind tunnel with persistent RH asymmetries, affect the location of moths arriving at the upwind end. The detection of RH differences could also play a role in orientation at large scales (from tens of meters to kilometers),

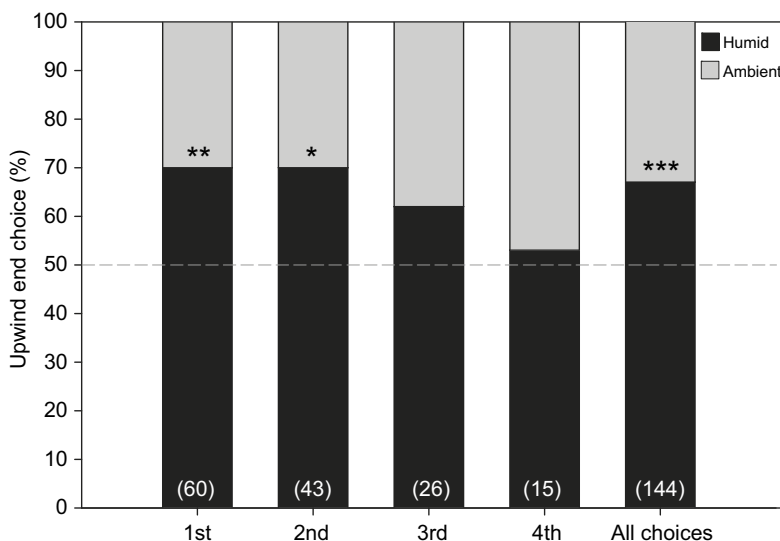


Fig. 2. Upwind flight responses in a flight tunnel without floral stimuli. Moths were significantly biased toward the humid side of the tunnel for their first and second choices when no odor was present. They did not display significant bias in their third or fourth choices. Overall, choices were significantly biased toward the humid side. * $P<0.005$; ** $P<0.001$; *** $P<0.0001$.

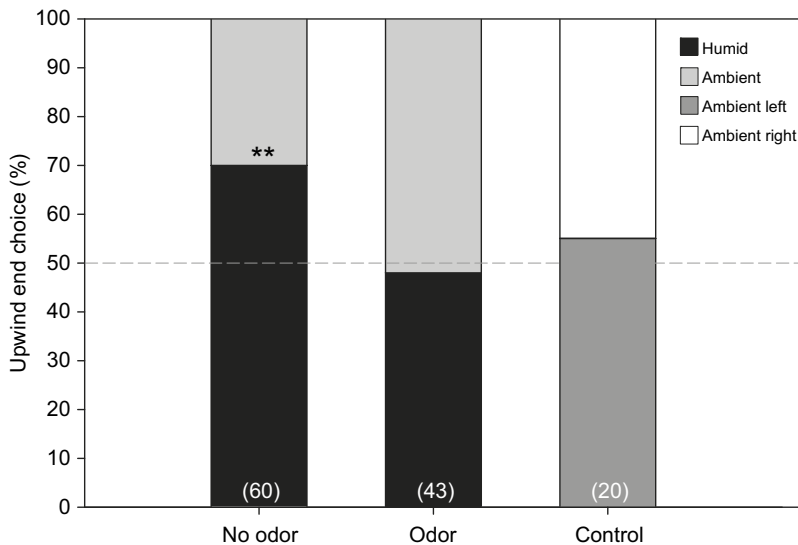


Fig. 3. Upwind flight responses with and without floral stimuli. The significant bias toward the humid side was not displayed when moths were exposed to floral stimuli (odor and visual display of artificial flowers on both sides of the upwind end of the tunnel. The right column shows responses when moths flew in the tunnel without added humidity or floral stimuli (i.e. side bias negative control). ** $P < 0.001$.

mediating behaviors that involve seasonal migrations, daily dispersion and habitat selection.

At smaller scales (meters to centimeters), *M. sexta* use floral scent to orient toward nectar sources, and these odors have been shown to effectively elicit foraging responses in still-air flight cages (Raguso and Willis, 2002), in wind tunnels with moving air (Goyret et al., 2007, 2008) and in natural, outdoor settings (Raguso and Willis, 2005). At much smaller scales (centimeters), the microhabitat surrounding flowers has elevated RH (over ambient conditions), which is correlated transiently with nectar concentration (Corbet, 1978; Corbet et al., 1979). We previously showed that hawkmoths of another species, *H. lineata*, preferentially probe at artificial flowers with an elevated RH when foraging in still-air flight cages scented with floral odors (von Arx et al., 2012). Thus, we asked whether the bias of *M. sexta* moths for more humid air observed under a non-foraging context (experiment 1) would persist under a foraging context provided by the presence of floral stimuli (experiment 2).

To approach this question, we designed experiment 2 as a replica of experiment 1 in which identical floral stimuli (visual and olfactory) were added to both upwind sides of the wind tunnel. If moths continued to orient towards the more humid side of the tunnel while demonstrating foraging behavior (i.e. probing at the artificial flowers), this would (1) indicate that foraging stimuli do not affect upwind orientation towards higher RH and (2) suggest that, under a foraging context, moths could be using RH as a distance floral profitability indicator. In contrast, if moths continued to fly towards the more humid side without showing foraging behaviors, it would suggest that floral stimuli have no effect on the behavioral state of moths that are orienting upwind using RH cues. Instead, moths in experiment 2 showed no side bias in their upwind flight, exhibiting clear foraging responses by approaching and probing artificial flowers, and thus showing overall longer residency times. These results suggest that the presence of floral stimuli triggered a foraging-specific behavioral state, in which mediation of upwind flight orientation by RH is overridden, probably, at this scale, by olfactory stimulation. This is consistent with strong stereotypical upwind flight orientation patterns of *M. sexta* in response to floral odors (as well as female pheromones and hostplant volatiles; Raguso and Willis, 2003). Nevertheless, the first flower probing tended to occur on flowers on the humid side. The question remains whether a close-range effect of odors and increased RH could have

contributed to this tendency, as shown in *H. lineata* moths (von Arx et al., 2012; see below).

Beyond eliciting a directed flight at the scale of meters, floral odors have been shown to elicit probing responses at the scale of centimeters (Raguso and Willis, 2002), in particular under conditions of poor illumination (Goyret and Yuan, 2015). The question of whether *M. sexta* moths utilize fine-scale spatial differences in RH as indices of individual floral profitability will require a flight cage experiment with either a pair or an array of artificial flowers, as performed by von Arx et al. (2012) using *H. lineata* moths.

Experiments probing the context-dependent use of sensory inputs provide insight into the integration of sensory information and its associated behavioral flexibility in animals that navigate dynamic environments. Because RH has several potential contextual meanings ranging from the presence of standing water to the existence of a patch of vegetation, a specific habitat or a humid air mass, a deeper understanding of RH as an informative sensory cue requires experiments that pair it with different kinds of stimuli, at appropriate spatial scales. Likewise, we expect the use of RH cues to be contingent upon the internal (physiological) state of the insect (e.g. osmotic stress, age or mating status; Willmer, 1988; Ryne et al., 2001; Saveer et al., 2012; Contreras et al., 2013) as well as external abiotic conditions that determine the efficacy of a specific sensory cue (Corbet et al., 1990; Leonard et al., 2011). Our results suggest that a hawkmoth dispersing from a seasonally dry habitat would show RH-guided anemotactic flight, especially if it were experiencing osmotic stress, which leads to mortality at a younger age (Contreras et al., 2013). Yet, hawkmoth flight is energetically demanding (Ziegler and Shultz, 1986) and, as we have shown here, their upwind flight orientation to RH cues can be disengaged by foraging responses to floral stimuli. Upon arriving at a more suitable habitat, the same moth could then use more-specific plant and floral cues (as well as local RH gradients) to locate nectar sources and host plants. Investigating this adaptive, contextual use of sensory cues should provide a more nuanced understanding of how insect pollinators and other animals navigate dynamic landscapes of sensory information.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: R.A.R., G.D., J.G.; Methodology: M.S.W., R.A.R., G.D., J.G.; Formal analysis: M.S.W., R.A.R., J.G.; Data curation: M.S.W., R.A.R., J.G.; Writing - original draft: M.S.W., R.A.R., J.G.; Writing - review & editing: R.A.R., G.D., J.G.; Supervision: R.A.R., J.G.; Funding acquisition: R.A.R., G.D., J.G.

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References

- Adler, L. S. and Bronstein, J. L.** (2004). Attracting antagonists : does floral nectar increase leaf herbivory? *Ecology* **85**, 1519-1526.
- Alarcón, R., Waser, N. M. and Ollerton, J.** (2008). Year-to-year variation in the topology of a plant-pollinator interaction network. *Oikos* **117**, 1796-1807.
- Angioy, A., Stensmyr, M. C., Urru, I., Puliafito, M., Collu, I. and Hansson, B. S.** (2004). Function of the heater: the dead horse arum revisited. *Proc. Biol. Sci.* **271**(Suppl), S13-S15.
- Bronstein, J. L., Huxman, T., Horvath, B., Farabee, M. and Davidowitz, G.** (2009). Reproductive biology of *Datura wrightii*: the benefits of a herbivorous pollinator. *Ann. Bot.* **103**, 1435-1443.
- Chittka, L. and Raine, N. E.** (2006). Recognition of flowers by pollinators. *Curr. Opin. Plant Biol.* **9**, 428-435.
- Clarke, D., Whitney, H., Sutton, G. and Robert, D.** (2013). Detection and learning of floral electric fields by bumblebees. *Science* **80**, 66-70.
- Contreras, H. L., Goyret, J., von Arx, M., Pierce, C. T., Bronstein, J. L., Raguso, R. A. and Davidowitz, G.** (2013). The effect of ambient humidity on the foraging behavior of the hawkmoth *Manduca sexta*. *J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol.* **199**: 1053-1063.
- Corbet, S. A.** (1978). Bee visits and the nectar of *Echium vulgare* L. and *Sinapis alba* L. *Ecol. Entomol.* **3**, 25-37.
- Corbet, S. A., Unwin, D. M. and Prys-Jones, O. E.** (1979). Humidity, nectar and insect visits to flowers, with special reference to *Crataegus*, *Tilia* and *Echium*. *Ecol. Entomol.* **4**, 9-22.
- Corbetta, M., Miezin, F., Dobmeyer, S., Shulman, G. and Petersen, S.** (1990). Attentional modulation of neural processing of shape, color, and velocity in humans. *Science* **248**, 1556-1559.
- Davidowitz, G.** (2002). Does precipitation variability increase from mesic to xeric biomes? *Global Ecol. Biogeogr.* **11**, 143-154.
- Gonzalez-Terrazas, T. P., Koblitz, J. C., Fleming, T. H., Medellín, R. A., Kalko, E. K. V., Schnitzler, H.-U. and Tschapka, M.** (2016). How nectar-feeding bats localize their food: echolocation behavior of *Leptonycteris yerbabuena* approaching cactus flowers. *PLoS One* **11**, e0163492.
- Goyret, J. and Raguso, R. A.** (2006). The role of mechanosensory input in flower handling efficiency and learning by *Manduca sexta*. *J. Exp. Biol.* **209**, 1585-1593.
- Goyret, J. and Yuan, M. L.** (2015). Influence of ambient illumination on the use of olfactory and visual signals by a nocturnal hawkmoth during close-range foraging. *Integr. Comp. Biol.* **55**, 486-494.
- Goyret, J., Markwell, P. M. and Raguso, R. A.** (2007). The effect of decoupling olfactory and visual stimuli on the foraging behavior of *Manduca sexta*. *J. Exp. Biol.* **210**, 1398-1405.
- Goyret, J., Markwell, P. M. and Raguso, R. A.** (2008). Context- and scale-dependent effects of floral CO₂ on nectar foraging by *Manduca sexta*. *Proc. Natl. Acad. Sci.* **105**, 4565-4570.
- Janzen, D. H.** (1984). Two ways to be a tropical big moth: Santa Rosa saturniids and sphingids. *Oxford Surv. Evol. Biol.* **1**, 85-144.
- Janzen, D. H.** (1987a). How moths pass the dry season in a costa rican dry forest. *Insect Sci. Its Appl.* **8**, 489-500.
- Janzen, D. H.** (1987b). When, and when not to leave. *Oikos* **49**, 241.
- Johnsen, S., Kelber, A., Warrant, E., Sweeney, A. M., Widder, E. A., Lee, R. L. and Hernández-andrés, J.** (2006). Crepuscular and nocturnal illumination and its effects on color perception by the nocturnal hawkmoth *Deilephila elpenor*. *J. Exp. Biol.* **209**, 789-800.
- Leonard, A. S., Dornhaus, A. and Papaj, D. R.** (2011). Forget-me-not- Complex floral displays, inter-signal interactions, and pollinator cognition. *Curr. Zool.* **57**, 215-224.
- Powell, J. A. and Brown, J. W.** (1990). Concentrations of lowland sphingid and noctuid moths at high mountain passes in eastern Mexico. *Biotropica* **22**, 316-319.
- Raguso, R. A.** (2004). Flowers as sensory billboards: progress towards an integrated understanding of floral advertisement. *Curr. Opin. Plant Biol.* **7**, 434-440.
- Raguso, R. A. and Willis, M. A.** (2002). Synergy between visual and olfactory cues in nectar feeding by naïve hawkmoths, *Manduca sexta*. *Anim. Behav.* **64**: 685-695.
- Raguso, R. A. and Willis, M. A.** (2003). Hawkmoth pollination in Arizona's Sonoran Desert: behavioral responses to floral traits. In *Butterflies: Ecology and Evolution Taking Flight* (ed. C. L. Boggs, W. B. Watt and P. R. Ehrlich). Chicago: The University of Chicago Press.
- Raguso, R. A. and Willis, M. A.** (2005). Synergy between visual and olfactory cues in nectar feeding by wild hawkmoths, *Manduca sexta*. *Anim. Behav.* **69**, 407-418.
- Ryne, C., Ekeberg, M., Olsson, P.-O. C., Valeur, P. G. and Löfstedt, C.** (2001). Water revisited: a powerful attractant for certain stored-product moths. *Entomol. Exp. Appl.* **103**, 99-103.
- Saveer, A. M., Kromann, S. H., Birgersson, G., Bengtsson, M., Lindblom, T., Balkenius, A., Hansson, B. S., Witzgall, P., Becher, P. G. and Ignell, R.** (2012). Floral to green: mating switches moth olfactory coding and preference. *Proc. R. Soc. B-Biological Sci.* **279**, 2314-2322.
- Simon, R., Holderied, M. W., Koch, C. U. and von Helversen, O.** (2011). Floral acoustics: conspicuous echoes of a dish-shaped leaf attract bat pollinators. *Science* **333**, 631-633.
- Stevenson, R. D., Corbo, K., Baca, L. B. and Le, Q. D.** (1995). Cage size and flight speed of the tobacco hawkmoth *manduca sexta*. *J. Exp. Biol.* **1672**, 1665-1672.
- Suzán, H., Nabhan, G. P. and Patten, D. T.** (1994). Nurse plant and floral biology of a rare night-blooming cactus. *Soc. Conserv. Biol.* **8**, 461-470.
- Unland, H. E., Houser, P. R., Shuttleworth, W. J. and Yang, Z. L.** (1996). Surface flux measurements and modeling at a semi-arid Sonoran Desert site. *Agric. For. Meteorology.* **82**, 119-153.
- von Arx, M., Goyret, J., Davidowitz, G. and Raguso, R.** (2012). Floral humidity as a reliable sensory cue for profitability assessment by nectar-foraging hawkmoths. *Proc. Natl. Acad. Sci. USA* **109**, 9471-9476.
- von Helversen, D., and von Helversen, O.** (2003). Object recognition by echolocation: a nectar-feeding bat exploiting the flowers of a rain forest vine. *J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol.* **189**, 327-336.
- Whitney, H. M., Dyer, A., Chittka, L., Rands, S. A. and Glover, B. J.** (2008). The interaction of temperature and sucrose concentration on foraging preferences in bumblebees. *Naturwissenschaften.* **95**, 845-850.
- Willmer, P. G.** (1988). The role of insect water balance in pollination ecology: *Xylocopa* and *Calotropis*. *Oecologia.* **76**, 430-438.
- Ziegler, R. and Shultz, M.** (1986). Regulation of carbohydrate metabolism during flight in *Manduca sexta*. *J. Insect Physiol.* **32**, 997-1001.