

Sensory Flexibility in Hawkmoth Foraging Behavior: Lessons from *Manduca sexta* and Other Species

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Introduction

Neuroethologists wish to understand how animals detect, integrate and perceive sensory stimuli relevant to complex behaviors, and how such processes are modified by physiological state and experience. The tobacco hornworm moth (*Manduca sexta*) is an excellent model organism for these purposes, due to its large size, rapid generation time and well-defined adult behaviors. Upwind-flight to sex pheromone (males), oviposition on appropriate hostplants (females) and feeding on floral nectar (both sexes) all require both olfaction and vision (Willis and Arbas, 1991). Although these behaviors feature sequences of stereotyped events, recent studies indicate plasticity in responses to critical sensory stimuli, depending upon the context in which they are perceived. Here we outline three themes of sensory flexibility or variation in hawkmoth behavior: (i) phylogenetic shifts in feeding niche, (ii) spatial and temporal scale of signal presentation and (iii) task specificity and signal redundancy.

Phylogenetic patterns and constraints

The diversity of feeding niches and diel activity patterns observed among the >1000 species of hawkmoths suggests that the relative importance of vision and olfaction should vary widely between species. Kitching and Cadiou (2000) suggest that long proboscides and adult feeding are derived conditions in the family Sphingidae. Crepuscular nectar-feeders like *M. sexta* use olfaction and vision to feed from flowers (Brantjes, 1978; Kawano *et al.*, 1995), whereas diurnal nectar-feeders like *Macroglossum stellatarum* find flowers using vision alone (Kelber, 1997). Hawkmoths that feed from fermenting sap (*Darapsa pholus*; Fleming, 1970) or bee honey (*Acherontia atropos*; Kitching, 2003) must rely heavily on olfactory cues to find food. Finally, species that are active day and night (*Hyles lineata*) or feed from flowers and rotting fruit (*Amphion floridensis*) (Raguso and Willis (2003) probably modify their behavior based on light intensity, food availability and prior experience.

Thus, inter-specific variation in sensory modality utilization should be expected in the Sphingidae. However, comparing behavior across species requires experimental parameters to be standardized (e.g. Chittka *et al.*, 2001). Daly and Smith (2000) classically conditioned *M. sexta* to single odors with sucrose and measured conditioned responses as cibarial pump muscle activity. When released, these moths flew up wind tunnels and extended their probosces to the conditioned stimulus (K.C. Daly *et al.*, unpublished data). Conversely, Kelber *et al.* (2003) showed with operant conditioning that *Deilephila elpenor* and *Hyles lineata* learn to probe at scentless colored targets associated with sugar rewards. Differences in experimental conditions and learning paradigms limit the extent to which the relative importance of olfactory and visual inputs to these species can be compared.

Spatial and temporal scale of signal presentation

Nectar foraging by hawkmoths involves behavioral responses to sensory inputs at different spatial scales, including distance orientation (>10 m), floral approach (<10 m), and, at a flower's threshold, proboscis extension and feeding. Raguso and Willis (2003) summarized the evidence for hawkmoth upwind flight to flower and fruit odors. Fragrance and visual contrast synergize floral approach and feeding by lab-reared and wild *M. sexta* (Raguso and Willis, 2002, 2005). Although Knoll's (1926) classic experiments indicated visual guidance of proboscis placement by *Hyles lineata livornica*, Desai and Raguso (2002) showed that *M. sexta* use visual contrast, tactile and gustatory cues to probe for nectar. Thus, sensory switching by *M. sexta* during nectar foraging is a consequence of the flowers' physical proximity and the sensory information they present.

Temporal components of floral signal presentation also impact *M. sexta*'s foraging behavior. All nectar-feeding animals assess the relative profitability of individual flowers or patches while foraging (Bell, 1986); one problem they encounter is variance in the correlation between floral signals and reward quality. Post-pollination fragrance- and color-change are too gradual to reliably indicate nectar absence to foraging moths in short-lived flowers (Eisikowitch and Lazar, 1987; Tollsten, 1993). Do other cues track nectar availability on the scale of minutes, rather than hours or days? Many night-blooming flowers show dramatic bud growth preceding anthesis, coupled with intensive nectar and fragrance production (Raguso and Willis, 2003). Such metabolic activity predicts the accumulation of floral CO₂, which Guenstein *et al.* (2004) have confirmed for *Datura wrightii*, a night-blooming plant favored by *M. sexta* as a nectar source in North America's Sonoran Desert. Most feeding by *M. sexta* occurs within the first hour after *Datura* flowers open, when repeated moth visits deplete both nectar and CO₂ concentrations (P. Guenstein, unpublished data). *Manduca sexta* sensitively detect CO₂ through labial pit organ receptors (Kent *et al.*, 1986), and naïve moths innately prefer scented paper flowers with 765 p.p.m. CO₂ over those with ambient levels, but preference is not maintained in the absence of nectar (Thom *et al.*, 2004). Thus, floral CO₂ may function as an olfactory nectar-guide for *M. sexta*. It remains unclear whether CO₂ is redundant, complementary or synergistic to other floral cues at different spatial scales or levels of experience.

Task specificity, sensory cues and context

In addition to nectar feeding, *M. sexta* and other hawkmoths drink water from puddles and flowing streams (Nabokov, 1947; Janzen, 1984). These behaviors satisfy different physiological requirements (flight fuel versus water balance), but both involve proboscis extension. How might the same behavior (proboscis extension) result from different combinations of sensory cues? Naïve *M. sexta* require both visual and olfactory cues for proboscis extension in a nectar-feeding

context, but escaped moths in the lab probe at faucets above wet sinks, suggesting that humidity and chrome are super-normal water-surface cues.

We designed a randomized block experiment in which naïve, starved *M. sexta* were exposed to an air stream of either dry or humidified air alone or pumped over a strongly scented *Magnolia grandiflora* flower, for a total of four treatments. Moths' responses were measured to only one stimulus. The null hypothesis that neither water vapor nor fragrance is sufficient to elicit proboscis extension was rejected for males ($\chi^2 = 9.0$, 3 df, $P = 0.02$) and females ($\chi^2 = 8.0$, $P < 0.05$). Water vapor alone and floral scent with dry air elicited proboscis extension in some cases, but their combination had an additive (male $\chi^2 = 0.62$, $P > 0.75$; female $\chi^2 = 1.7$, $P > 0.5$), rather than synergistic effect (male $\chi^2 = 46.05$, $P < 0.001$; female $\chi^2 = 43.06$, $P < 0.001$). Perhaps fragrance and water vapor are functionally redundant cues that remain contextually distinct under field conditions. Alternatively, moth responses to floral scent may have been artifacts due to water vapor or CO₂ in *Magnolia* floral headspace. Further experiments are needed to decouple these cues by either desiccating the fragrant air or using essential oils with versus without humidified air or CO₂.

Here we have focused on sequences of behavioral *events* that comprise feeding, but we know much less about the factors that influence the motivational *state* that precedes these events (Martin and Bateson, 1993). A balanced experimental design in which starvation, prior feeding, age and mating status are manipulated would provide insight into what motivates moths to respond to the sensory signals that modulate feeding and drinking behavior.

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