Why do hawkmoths like nitrogenous volatiles? Exploring the behavioral relevance of "animalic notes" in white floral blends.

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Night-blooming plants with white, tubular flowers have evolved worldwide in association with long-tongued hawkmoths as pollinators. These plants include the wild progenitors of jasmine, gardenia, honeysuckle, tuberose and Easter lily, whose sweet, penetrating fragrances have long been important to the flavor and fragrance industry. The pioneering studies of Kaiser (1) established a nearly universal pattern of chemical composition for such plants, whose "white floral olfactory image" generally includes oxygenated terpenoids (linalool, nerolidol, farnesols), aromatic esters, alcohols and aldehydes, and nitrogenous compounds, including indole, aldoximes and associated nitro and nitrile compounds. Subsequent surveys of hawkmoth pollinated plant guilds have largely confirmed these patterns across diverse desert, grassland and rainforest environments (2-4). Additional studies have identified other compounds with fecal odors (e.g. cresols) in moth pollinated plants (5,6). Why do such cloyingly sweet floral blends often include "animalic notes" which are so strikingly unpleasant to human perception? What are the biological functions of such compounds embedded within the matrix of otherwise sweet white floral blends? Here we report chemical ecological bioassays performed on *Oenothera flava* (the yellow evening primrose) and *Ipomopsis tenuituba* (slender tube skyrocket), two wild plant species with white, night blooming flowers pollinated by hawkmoths in western North America. *Oenothera flava*, like many evening primroses, emits a powerful fragrance dominated by 2-methyl butyraldoxime, 3-methyl butyraldoxime, their associated nitriles and methyl nicotinate. *Ipomopsis tenuituba* emits a complex blend of *E,E*-farnesol, sesquiterpene hydrocarbons and indole. Each species has a closest relative that is not hawkmoth pollinated – either due to self-pollination or hummingbird pollination. We used a laboratory colony of the white lined sphinx moth (*Hyles lineata*), the natural pollinator of these plant species, as a source of flower-naïve moths for behavioral assays. Our experiments were designed to measure the innate preferences and foraging choices of starved, flower-naïve moths to nitrogenous volatiles in a nectar-rewarding context. In each case we observed a significant innate preference of the moths for scented flowers over their less strongly scented relatives, with fewer probing responses and longer latency to respond in the absence of nitrogenous volatiles. Low responses were reversed to some extent when physiologically relevant dosages of indole were added to red, hummingbird pollinated flowers of *Ipomopsis aggregata*, although responses were strongest when flowers also were colored white. Similarly, moths were innately attracted to yellow artificial flowers modeled after those of *Oenothera flava*, but their attraction increased when relevant dosages of 3-methyl butyraldoxime were added to the flowers. Interestingly, although moth antennae respond equally to 2-methyl and 3-methyl butyraldoxime, the moths showed no behavioral responses to the 2-methyl isomer. Given the independent evidence that another species of hawkmoth (*Manduca sexta*) responds behaviorally to blends of linalool, benzaldehyde and benzyl alcohol (7), it remains unclear why night blooming flowers with these compounds also include nitrogenous volatiles. We suspect that most "white floral" blends include either redundant

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or synergistic combinations of volatile compounds, perhaps to attract several moth species as pollinators, which could constitute a reproductive assurance strategy. Under different ecological contexts, aldoximes are emitted from herbivore induced foliage of poplar (*Populus nigra*) (8), just as indole is one of a suite of vegetative volatiles emitted by herbivore-induced maize (*Zea mays*)(9). Furthermore, indole often is emitted by flowers that mimic feces or carrion (10). The fact that these compounds are derived from essential amino acids, and are by-products of protein degradation may explain why they generally evoke negative responses in human olfaction, in stark contrast to the sweet, pleasant hedonic valence of other volatiles common to the “white floral” image.

1. Kaiser, R., 1993, The scent of orchids: olfactory and chemical investigations, Elsevier.
2. Knudsen, J.T.; Tollsten, L. Bot. J. Linn. Soc., 1993, **113**, 263-284.
3. Raguso, R.A.; Henzel, C.; Buchmann, S.L.; Nabhan, G.P., Int. J. Plant. Sci., 2003, **164**, 877-892.
4. Raguso, R.A.; Levin, R.A.; Foose, S.E.; Holmberg, M.W; McDade, L.A., Phytochem., 2003, **63**, 265-284.
5. Dobson, H.E.; Arroyo, J.; Bergström, L.G.; Groth, I., Biochem. Syst. Ecol. 1997, **25**, 685-706.
6. Chess, S.K.; Raguso, R.A.; LeBuhn, G., Am. J. Bot., 2008, **95**, 1652-1659.
7. Riffell, J.A.; Lei, H.; Christensen, T.A.; Hildebrand, J.G., Curr. Biol., 2009, **19**, 335-340.
8. Irmisch, S.; Unsicker, S.B.; Gershenzon, J.; Köllner, T.G., Pl. Sig. Behav. 2013, **8**, 327640.
9. Erb, M.; Veyrat, N.; Robert, C.A.; Xu, H.; Frey, M.; Ton, J.; Turlings, T.C.J., Nature Comm. 2015, **6**, 6273.
10. Jürgens, A.; Wee, S.L.; Shuttleworth, A.; Johnson, S.D., Ecol. Lett. 2013, **16**, 1157-1167.

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