In situ modeling of multimodal floral cues attracting wild pollinators across environments

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With more than 80% of flowering plant species specialized for animal pollination, understanding how wild pollinators utilize resources across environments can encourage efficient planting and maintenance strategies to maximize pollination and establish resilience in the face of environmental change. A fundamental question is how generalist pollinators recognize "flower objects" in vastly different ecologies and environments. On one hand, pollinators could employ a specific set of floral cues regardless of environment. Alternatively, wild pollinators could recognize an exclusive signature of cues unique to each environment or flower species. Hoverflies, which are found across the globe, are one of the most ecologically important alternative pollinators after bees and bumblebees. Here, we have exploited their cosmopolitan status to understand how wild pollinator preferences change across different continents. Without employing any a priori assumptions concerning the floral cues, we measured, predicted, and finally artificially recreated multimodal cues from individual flowers visited by hoverflies in three different environments (hemiboreal, alpine, and tropical) using a field-based methodology. We found that although "flower signatures" were unique for each environment, some multimodal lures were ubiquitously attractive, despite not carrying any reward, or resembling real flowers. While it was unexpected that cue combinations found in real flowers were not necessary, the robustness of our lures across insect species and ecologies could reflect a general strategy of resource identification for generalist pollinators. Our results provide insights into how cosmopolitan pollinators such as hoverflies identify flowers and offer specific ecologically based cues and strategies for attracting pollinators across diverse environments.

multimodal factors | categorization | syndrome | hoverfly | multivariate

The majority of the world's flowering plant species rely on animal pollinators (1), yet more than 40% of our invertebrate pollinators are currently threatened by habitat loss, environmental change, pesticide use, disease, and several other factors (2). Preserving pollination in the face of decreasing numbers requires understanding the natural ecology of wild pollinators (3). Indeed, identifying specific strategies that attract wild pollinators is a crucial (4, 5), yet often overlooked, aspect of pollination. Our world's ecosystems are also experiencing rapid change due to changes in climate and the so-called "Anthropocene Era" galvanized by human effects on our planet (6). Combined with the impending effects of environmental change, we are faced with a double-edged problem: We need to understand why and how pollinators utilize certain sources and then identify how these relationships change across different environments. One major benefit to preserving cosmopolitan pollinators across environments could be to bolster ecosystem services over space and time (7).

Hoverflies, which are found across the globe, are one of the most important alternative pollinators after bees and bumblebees (8, 9). The agriculturally important marmalade hoverfly, *Episyrphus balteatus*, can be found in tropical Bangalore and alpine Sikkim, India, and in hemiboreal Uppsala, Sweden (Fig. 1*A*) (10–13). Meanwhile, the drone fly, *Eristalis tenax*, is found in both Uppsala and Sikkim (Fig. 1*A*), and the genus is spread across Holarctic, Asian, Neotropical, and Ethiopian regions (10–14). These pollinators thus provide an ideal model to assess how wild pollinator preferences change across different environments.

Flowers may use scent, color, morphology, or local CO_2 and humidity gradients to attract pollinators (15–18). How do cosmopolitan pollinators recognize and distinguish suitable "flower objects" in vastly different ecologies and environments? Many wild pollinators, like hoverflies, are generalists and feed from several flower species, suggesting that they must categorize suitable flower objects rather than individual species. Hoverflies have an innate preference for yellow (19–21), but are also attracted to blue (22), pink (23), and purple (22, 24) flowers, as well as by olfactory cues (23). Since interactions between olfaction and vision enhance floral attraction for many pollinators (25, 26), it is likely that hoverflies also use multimodal factors for recognition of flower objects. Historically, pollination literature was dominated by the concept of syndromes, namely specific flower shapes, colors, and scent characteristics associated with particular animal pollinators (27). However, recent studies have focused on community-level characteristics such as pollination

Significance

The coevolution of flowers and pollinators is well known, but how generalist pollinators identify suitable flowers across environments and flower species is not well understood. Hoverflies, which are found across the globe, are one of the most important alternative pollinators after bees and bumblebees. Here we measured, predicted, and finally recreated multimodal cues from individual flowers visited by hoverflies in three different environments (hemiboreal, alpine, and tropical). We found that although "flower signatures" were unique for each environment, some cues were ubiquitously attractive, despite not resembling cue combinations from real flowers. Our results provide unique insights into how a cosmopolitan pollinator identifies flower objects across environments, which has important implications for our understanding of pollination as a global ecological service.

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Data deposition: The entire datasets for real flowers and for artificial lures can be found in DataDryad, https://doi.org/10.5061/dryad.s7jb3.

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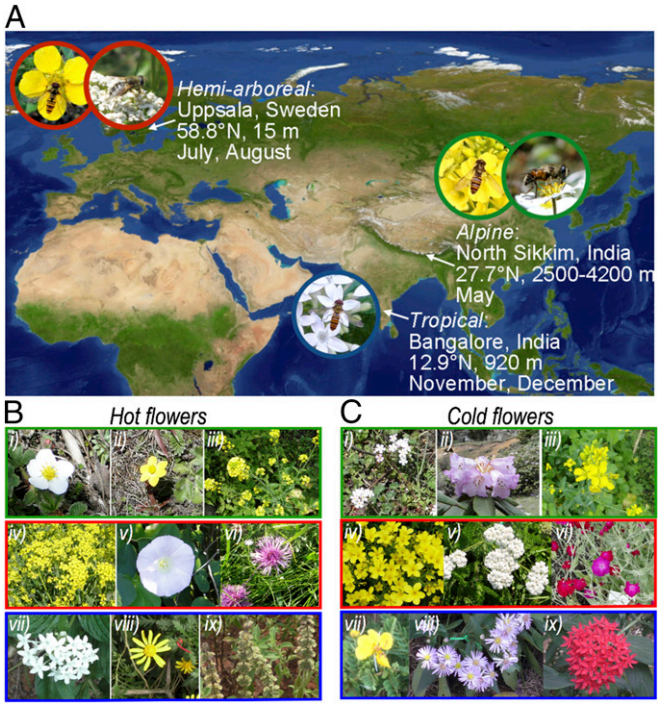

Fig. 1. Observing flowers in three climate regions. (*A*) Location of the three regions used for collection of data at specified dates (coinciding with hoverfly peak seasons). Region color coding is used in all figures. Images show *Episyrphus* sp. and *Eristalis* sp., respectively. Examples of (*B*) Hot and (*C*) Cold flowers from the three regions as indicated by colored frames. Flower species are listed in *SI Appendix*, Table S1.

landscapes, webs, networks (27), and magnet species (28, 29), due in large part to the apparent "paradox" of seemingly specialist flowers being visited by many types of pollinators and vice versa (27). As such, while specialist pollinator syndromes are relatively well supported, the concept of a generalist syndrome encompassing several flower species is often attributed to features that increase the likelihood of encounter or apparency (cf. ref. 27).

On one hand, generalist pollinators could employ a specific syndrome of cues present across several flower species to identify flower objects regardless of environment or ecology. Alternatively, they could recognize an exclusive signature of specific cues unique to each environment and impacted by local community structure. Flowers have evolved scent, color, and pattern together with the anatomical, behavioral, physiological, and ecological features of their pollinators, such as peaks in color vision or olfactory sensitivity (27, 30–33). In turn, these cues can be impacted by phylogenetic, pleiotropic (e.g., carotenoids as accessory pigments for chlorophyll), exaptative (e.g., pigmentation aiding plant survival), and ecological constraints on the flowers themselves (33). Multimodal interactions between olfactory and visual cues could independently or interactively enhance the apparency or context of individual cues (29) and also associative learning for flower recognition (26, 34) by exploiting parallel processing, sensory bias and overload, and the perceptual variability achieved with multiple cues (35). These lines of evidence suggest that generalist pollinators could also be attracted to syndromes of floral cues (i.e., color and scent combinations). Unfortunately, there has been little systematic effort to identify multimodal combinations that could increase attention, context, or exploit perceptual bias across multiple geographical environments and ecologies.

To explore these possibilities for "flower object identification" in a generalist pollinator, we developed a unique in situ methodology (*SI Appendix*, Fig. S1) to quantify the cues that hoverflies use to categorize suitable flowers in three different environments, including hemiboreal Uppsala, alpine North Sikkim, and tropical Bangalore. Without employing any *a priori* assumptions on the nature of the cues, we measured multiple phenotypic characters likely associated with pollinator attraction to flowers (27), including ratio and composition of volatiles, reflected wavelengths, absolute size and shape of corolla and inflorescence, humidity, temperature, and CO_2 emission. We then used multivariate data analysis to predict combinations of cues that were attractive, or less attractive, to hoverflies in each environment, while being agnostic to the flower species. Finally, we created artificial lures exhibiting combinations of floral cues predicted by our multivariate data analysis, placed the lures in the same three environments and temporal and spatial contexts, and recorded hoverfly visits. This allowed us to disentangle individual vs. community effects on the apparency or context of selected cues.

Results

To address wild pollinator preference across climates, we sampled floral phenotypic and abiotic data resulting in >1,000,000 data points from 153 individual flowers in three different environments, of which 112 were "Hot" (i.e., visited by hoverflies). These points consisted of illuminance, reflected UV and visual wavelengths (308–760 nm), absolute flower size (area, maximum and minimum ferret), corolla circularity and type, inflorescence type, volatile identities and ratios (up to 96 volatiles), humidity, and CO_2 measurements. Our observation of attractive (Hot, Fig. 1*B*, flower species listed in *SI Appendix*, Table S1) and less attractive, although not necessarily repellent (Cold, Fig. 1*C*) flowers in the immediate vicinity of each other (*SI Appendix*, Fig. S2*A*) indicates that it would be difficult to conclude that hoverfly flower choice is based on visual cues alone. For example, composite flowers could be either Hot (Fig. 1*B*, *vi* and *viii*) or Cold (Fig. 1*C*, *viii*), and the same flower species could be both Hot and Cold (Fig. 1*B*, *iii* and Fig. 1*C*, *iii*). Yellow flowers, which are often listed as attractive for hoverflies (19–21, 27), were also found in both categories (Fig. 1*B*, *iii* and Fig. 1*C*, *iv*). In total, 16 angiosperm families belonging to 13 orders of flowers were sampled from the three regions (*SI Appendix*, Table S2). Overall, the variation of species suggests the potential for large variations in floral signature among and between the different geographic regions.

We developed techniques for local sampling of spectral (Fig. 2*A*), abiotic (Fig. 2*B*), and volatile factors (Fig. 2*C* and *SI Appendix*, Fig. S2) from individual flowers in the field, without assuming any cue's relative importance for hoverfly choice. We used photographs to quantify flower size and circularity (Fig. 2*A*, *i–iv*), manually score corolla shape (*SI Appendix*, Table S3), and account for flower abundance in the immediate vicinity of the measured flower (*SI Appendix*, Table S4). We also determined the volatile signature emitted by each flower using a recently developed technique for "snapshot" sampling of volatiles in the field at any moment (36) (*SI Appendix*, Fig. S2). By analyzing all samples with GC-MS (Fig. 2*C*, black data show a Hot and gray data a Cold *Rhododendron campanulatum* flower), we identified 96 different compounds from the 153 samples (*SI Appendix*, Table S5), constituting a variety of chemical classes, but only a small number of compounds were significantly different between Hot and Cold flowers (*SI Appendix*, Fig. S3).

We used multivariate data analysis to predict combinations of attractive or less attractive cues for hoverflies in each environment. We performed separate multivariate data analyses (MVA) for each region, first employing principal component analysis (PCA) to reduce color information, and then orthogonal partial least squares (OPLS) analysis to identify parameters associated with Hot or Cold flowers (Fig. 3 *A–F* and *SI Appendix*, Fig. S4). We pooled data from flowers visited by any hoverfly genus, as their identity did not appear to influence the results (*SI Appendix*, Fig. S5). From the OPLS, we identified variables with a loading score (*P* value) greater than 0.1 (dashed lines, Fig. 3 *A–F*) and error bars that did not cross 0, and used these to model Hot and Cold floral signatures (colored bars, Fig. 3 *A–F*). As we were not attempting to find the most attractive flower species, and we often found both Hot and Cold flowers of the same

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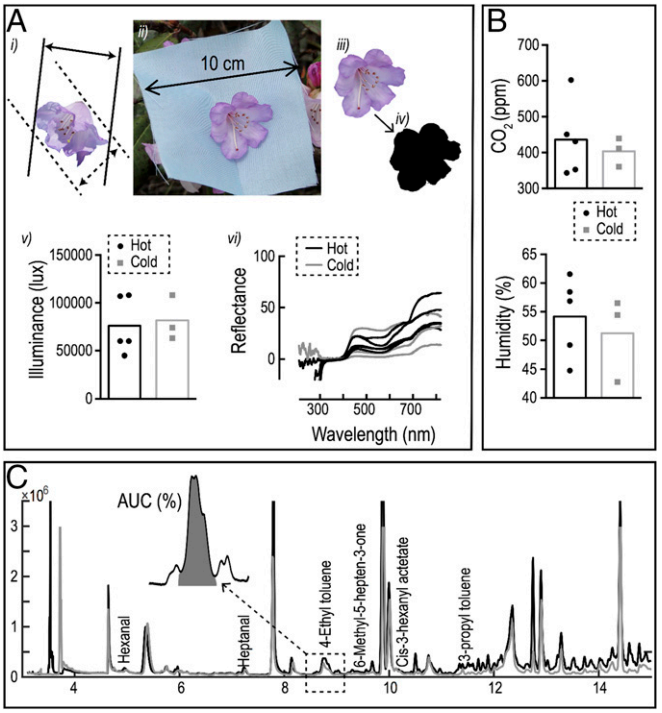

Fig. 2. Measuring multimodal variables. (*A*) Data from five Hot *R. campanulatum* flowers (*i*) from North Sikkim (black in *v* and *vi*), and data for three Cold *R. campanulatum* flowers (*iii*) (gray in *v* and *vi*). We used photographs for size and shape quantification (*i–iv*), lux meter for illuminance (*v*, mean ± SD), and spectrophotometer for color (*vi*, eight individual measurements shown). Projected flower size and circularity was quantified by counting black and white pixels (*ii–iv*), using known size of background cloth. (*B*) CO_2 and humidity were sampled within each corolla, using a custom abiotic sensor (mean ± SD). (*C*) Two sample traces of a Hot (black) and a Cold (gray) *R. campanulatum*. Volatile data were collected using PDMS and analyzed by GC-MS. Floral peaks were identified by comparison with the blank spectra (*SI Appendix*, Fig. S2*B*). Area under each curve (AUC, magnified *Inset*) was used to calculate relative ratios of total floral volatiles.

species (*SI Appendix*, Tables S1 and S2), species information was not included in the analysis. Instead, we were investigating attractive combinations of floral phenotypic and abiotic cues, as they were detected in situ.

In all cases, hoverflies were predicted to use multimodal cues for identifying suitable flower signatures (colored bars, Fig. 3 *A–F*). Some parameters were found in all three regions, but sometimes had opposite effects on hoverfly visitation. This indicates that hoverflies use unique multimodal flower signatures in each region and suggests that environmental or ecological changes in the saliency of these signatures could also occur. These parameters were not affected by parameter randomization or by specific hoverfly genera (i.e., *Episyrphus* sp., *SI Appendix*, Fig. S5), suggesting that they are neither a result of chance nor a function of hoverfly genera sampled at each location.

We next used the predicted signatures for each region (Fig. 3 *A–F*) to create artificial lures. Often, Hot and Cold cues were not mutually exclusive (Fig. 3 *G–J*). Nevertheless, we had to choose some color, shape, size, and odor for all lures (Fig. 4*A*) and for parsimony chose the opposite of the predicted cue if no variable was available. In Uppsala, for example, there were more variables associated with Hot signatures (Fig. 3*C*) than Cold (Fig. 3*D*), but since many contained information about size, shape, and color (blue and red data, Fig. 3*C*), we predicted that they would impact Cold signatures, too. For example, the Bangalore Cold lure (Fig. 4*A*, *vi*) consisted of stellate (blue bar, Fig. 3*F*), green (red bar, Fig. 3*F*), and comparatively large (blue bars, Fig. 3*F*) clustered corollas (blue bar, Fig. 3*F*) with nonanal and decanal (green bars, Fig. 3*F*) in a microcentrifuge tube. We used similar reasoning for the other lures, and, where we had no inflorescence information from the OPLS, lures were placed individually (Fig. 4*A*). We additionally created a negative control (an odorless black circle, Fig. 4*A*, *viii*) and a unique positive control for each region exhibiting color, size, shape, and odor profiles from an exclusively Hot flower species found in each environment (e.g., *Potentilla fruticosa* for Uppsala, Fig. 4*A*, *vii*). Note that there was no sugar or pollen reward in the lures to reduce risk of individual hoverflies returning to the lures, or learning new flower signatures.

Finally, we placed the lures in the same three environments and recorded hoverfly visits in the same spatial and temporal locations where the floral data were collected the previous year (Fig. 4*B* and *SI Appendix*, Fig. S6). In total, our lures attracted 150 visits in Sikkim, 112 in Uppsala, and 146 in Bangalore. Our data show that visits to the eight lures were significantly different from chance (i.e., equal visits to all lures, χ^2 test, $P < 0.0001$ in all regions; $P < 0.001$ in all regions when excluding visits to the real flower) and that the lures were as attractive as a real flower in both Sikkim and Uppsala (Fig. 4*B*). Overall, our data show few hoverfly visits to the negative control, but other insects did visit it (*SI Appendix*, Fig. S6). For a detailed breakdown of visits by certain hoverfly species, see *SI Appendix*, Fig. S7. Cold lures exhibited varying degrees of attraction both within and across environments, implying that the MVA was not necessarily able to predict less attractive Cold signatures. We thus conclude that it is possible to use OPLS multimodal signatures to create artificial lures that are attractive to pollinators within and across environments, despite the lures not mimicking real flowers or manipulations of real flowers (as in, e.g., refs. 25 and 37–39). This is important as the specific combinations of color, shape, size, and odor in our lures (*SI Appendix*, Fig. S8) do not exist in the natural environment.

Discussion

Our work offers a field-based methodology to examine cues involved in insect pollination across different environments that can be useful for other studies assessing how environmental and ecological changes affect plant and animal interactions. While we cannot confirm hoverfly perception of the floral cues, all measured cues were quantified in situ in the field, whereas previous analyses have often been laboratory-based, such as recording flower color under a xenon lamp (e.g., ref. 40). When measured in the field, cue variation in our lures across conditions was striking (*SI Appendix*, Fig. S8), suggesting that hoverfly sensory systems, or indeed those of any pollinator, must compensate for such variation when locating a flower in nature.

Importantly, our methodology was agnostic to flower species, and indeed, the same species could be found among both Hot and Cold samples (Figs. 1 *B* and *C* and 2). Furthermore, we employed no a priori assumptions about each cue's relative importance. Our resulting artificial lures (Fig. 4*A*) were solely based on the variables predicted from the MVA (Fig. 3) rather than attempting to recreate or modify real flowers or presume hoverfly perception. This is radically different from most previous work using artificial flowers (25, 37) or modifications of real flowers (e.g., ref. 38). The MVA (Fig. 3 *A–F*) was generally able to predict attractive signatures, but was less effective at predicting nonattractive signatures (Fig. 4*B*). While we controlled for local abiotic and biotic factors that could affect attractiveness (i.e., microclimate or community effects), the model could have been impacted by an inability to identify true negatives in the field, the lack of mutually exclusive cues between Hot and Cold flowers (Fig. 3 *G–J*) or unmeasured factors (27) such as flower microstructure, movement, or pollen and nectar content, which could have reduced our ability to predict nonattractive signatures.

Our use of artificial lures also allowed us to assess wild pollinator attraction to floral cues across environments without confounding effects of environment on floral biology and floral cues. Some signatures, such as the predicted Bangalore Hot lure, were attractive only in their own environment, while the predicted Uppsala Hot lure was attractive everywhere except Uppsala (Fig. 4*B*). These differences highlight the importance

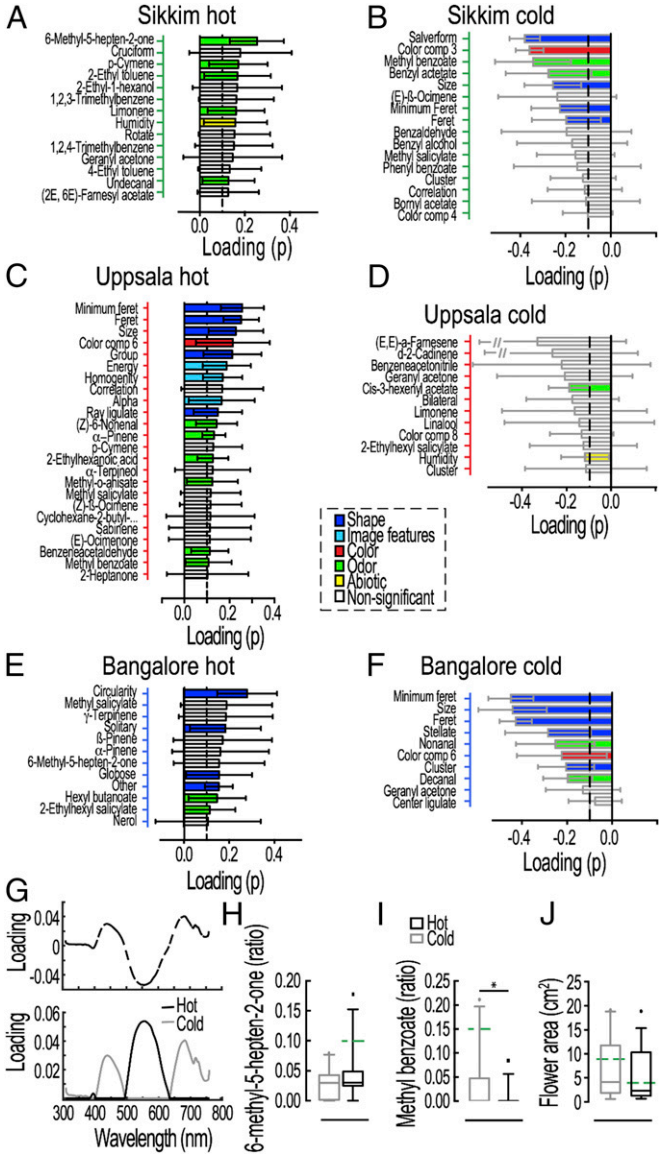

Fig. 3. Modeling Hot and Cold flowers across regions. (*A*) Section of OPLS output for flowers from alpine North Sikkim, showing the positive loadings (p) for Hot parameters. Cues with loadings above 0.1 and an error bar not crossing the midline are color coded by type (see color key). (*B*) OPLS output for Cold flowers from Sikkim at $P < -0.1$. (*C*) OPLS output for Hot and (*D*) Cold flowers from Uppsala. (*E*) Hot and (*F*) Cold flowers from Bangalore. (*G*) Loading scores for color component 3 from Cold Sikkim flowers (red, *B*). Hot color spectrum (black, *Bottom*), and Cold spectrum (gray, *Bottom*) created by separating the color component into positive and negative loadings. (*H*) Relative ratios of 6-methyl-5-hepten-2-one measured in Hot (black) and Cold (gray) flowers in Sikkim. Green dashed line shows ratio used for Hot lures. (*I*) Relative ratios of methyl benzoate in Hot (black) and Cold (gray) Sikkim flowers with green dashed line indicating ratio used in Cold lure. (*J*) Corolla size (area) for Sikkim Hot (black) and Cold (gray) flower with green dashed lines for flower sizes used for lures.

of both individual and community effects of floral traits on the apparency or context of selected cues. For example, while our selection of Hot and Cold flowers attempted to decouple community effects, it is possible that the attractive observations, particularly in Sweden, may still have been impacted by pollination webs, networks, or magnet effects not measured here and therefore not present in our artificial lures. Nevertheless, our results also highlight the potential for hoverfly syndromes (myophily), particularly with our Sikkim lure, which was attractive in all tested environments. Hoverflies are known to exhibit landmark association, specific visitation patterns, and floral constancy (27). While hoverflies are suggested to overlap with bees in floral choice and constancy (27), there are cases of potential selection for floral traits based on hoverfly visitation (41, 42). In Sikkim, hoverflies and other flies are the predominant pollinators, as bees, birds, and other animals are uncommon at high elevations (43, 44). There could thus be strong selection on Himalayan flowers to be attractive to hoverflies, their major pollinators.

It is known that pollinators are selective to floral traits and that floral signals are essential for the assessment of flowers (45). As such, it was unexpected that our lures did not need to replicate specific combinations of cues found in real flowers (e.g., the smell and color of a rose). Instead, our results show that these generalist pollinators do not require real flowers, but can be attracted to combinations of cues obtained from several attractive flowers. These combinations were not random or likely unimodal, as not all of our lures with similar characteristics (e.g., blue or yellow color) were equally attractive. This result is in contrast to previous studies replicating real floral cue combinations (25, 28, 29) and implies that generalist pollinators could potentially select from a series of multimodal cues as a form of independent, rather than interactive, syndrome (29). This strategy would also allow pollinators to locate suitable resources in unknown environments and contexts. Given the attractiveness of our lures across insect species (*SI Appendix*, Figs. S6 and S7), our results might reveal the potential for "generalist syndromes," particularly when the predictions were made by observing several species (*SI Appendix*, Table S2). Further studies parsing the relative attraction of multimodal cues to both naive and experienced pollinators can help us to unravel the role of pollinator and floral ecology across environments.

This study provides unique strategies for understanding wild pollinator preference. The robustness of our lures (e.g., the

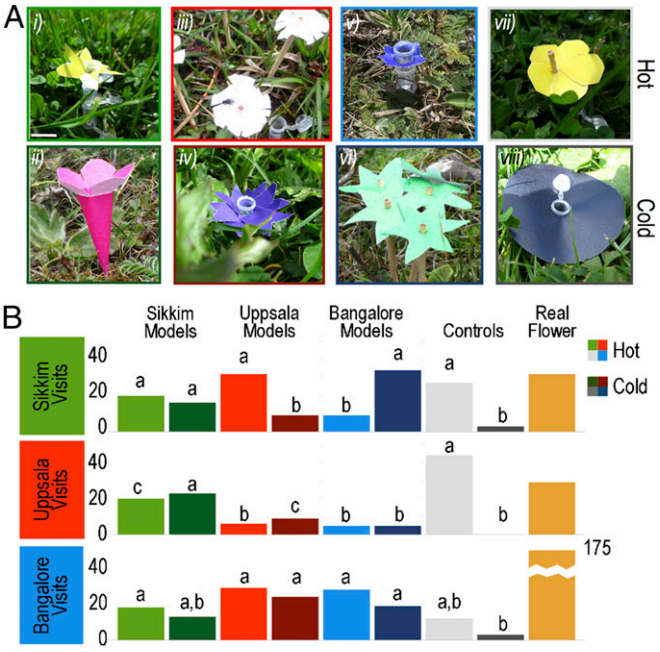

Fig. 4. Assessing the variables in situ. (*A*) Artificial lures created for each region (as color coded), with Hot flower lures in the *Top* row and Cold lures in the *Bottom* row. (*vii*) The Uppsala positive control, mimicking *P. fruticosa*. (*viii*) The negative control, used in all regions. (Scale bar, 1 cm.) (*B*) Total number of hoverfly visits recorded in the three regions compared with positive and negative controls (Fisher's exact test, $P < 0.05$). "Real flower" indicates visits to a natural flower within 90 cm of the lures.

Himalayan signatures) across communities and climates suggests that these cues, or flowers exhibiting these cues, could also increase the attractiveness of habitats such as agricultural fields over different ecologies and geographies. These cues must now be tested in large-scale field trials to assess their efficacy. Our methodology and large-scale data on wild pollinator preferences could also be beneficial for current efforts to increase habitat and forage resources in and around agricultural areas for pollinators (1), and could be used in many other ecological studies that analyze changes in resource use across environments. Nonetheless, since our results also suggest that wild pollinators rely only on a small number of cues, drastic changes in flower phenology due to habitat disruption, environmental change, or monocultures could make it difficult for wild pollinators to locate suitable pollination sites. This is especially important considering that the current study sampled an extremely reduced subset of all flowers in each region. As such, consideration should be taken while planting crops and gardens to retain the consistency of the floral signal. In addition, the region-specific differences in attractive floral signatures also suggest that climate, habitat, and geography play an important role in shaping plant–pollinator relationships. Therefore, there may not be a "one size fits all" answer to attracting pollinators across all environments. Our results provide important insights about the impact of climate and environment on plant–animal interactions. Further experiments directly assessing the plasticity of pollinator choice under ecological or environmental variation are needed to test these predictions and to understand the ecology of alternate pollinators in the face of our ever-changing environments.

Materials and Methods

Locations. We investigated hoverfly flower visitation in three environments selected for differences in climate, altitude, and hoverfly presence (e.g., refs. 10 and 11): (*i*) alpine North Sikkim, India, at altitudes ranging from 2,300 m (Chapten) to 4,200 m (Chopta valley) during May 10–15, 2015 and May 6–12, 2016; (*ii*) hemiboreal Uppsala, Sweden, at an altitude of 29 m during July 24–August 7, 2015 and August 14–24, 2016; (*iii*) tropical Bangalore, India, at 1,500 m altitude during November 5–December 8, 2015 and November 22–December 19, 2016. The 2015 season comprised data collection from individual real flowers, and the 2016 season the attractiveness of artificial lures. In all locations, both cultivated gardens and wild flower patches were measured to provide different floral communities (*SI Appendix*, Table S2). While agnostic to flower and hoverfly species, observations focused on hoverflies found in multiple regions, specifically *Eristalis* sp. and *E. balteatus*. All data were collected at the time of day and year for hoverfly activity at that location, with floral species dictated by hoverfly choice. We collected data from 57 real flowers in Sikkim (33 Hot and 24 Cold), 53 in Uppsala (43 Hot and 10 Cold), and 43 in Bangalore (36 Hot and 7 Cold). A flower was classified as Hot (Fig. 1*B*) if a hoverfly landed on it, or Cold (Fig. 1C) if the flower was in the immediate vicinity of Hot flowers (<2 m, *SI Appendix*, Fig. S2*A*), but no hoverflies approached during the observation time (30 min–3 h as determined by weather conditions and cue collection). Hot and Cold flowers, which could be from the same species or even plant (e.g., *Rhododendron* sp.), were selected for relative proximity to avoid local biotic and abiotic effects or plant–pollinator landscape issues (27) related to spatial or temporal webs, networks, or matrices that could affect attraction (*SI Appendix*, Fig. S2*A*). Due to these stringent guidelines, not all Hot flowers were accompanied by a corresponding Cold flower unless we could confirm a lack of visitation, but all Cold flowers were selected as a function of Hot flowers. We quantified data from flowers visited by any hoverfly, but Hot flowers were visited by *Eristalis* (22 of 33 in Sikkim, 31 of 43 in Uppsala) and *Episyrphus* (2 of 33 in Sikkim, 4 of 43 in Uppsala, 31 of 43 in Bangalore).

Visual Cues. We used a LM-120 light meter (Amprobe) to measure local, ambient illuminance. Photographs of each flower were obtained with a Sony DSC-HX1 with and without a 10- × 10-cm dull gray fabric collar around the flower. Corolla shapes were manually scored (*SI Appendix*, Tables S3), as was the relative abundance of conspecific flowers (*SI Appendix*, Tables S4). We quantified the surface area of each flower as seen by an approaching hoverfly using Fiji software (46) by first removing the background collar (Fig. 2*A*, *ii* and *iii*) and converting the flower pixels to black and white (Fig. 2*A*, *iv*) to quantify the black pixels. These were converted to flower area in square centimeters by comparison with the pixels corresponding to the background fabric of known size (Fig. 2*A*, *ii*). We used Fiji software (46) to calculate Feret's diameter (solid lines, Fig. 2*A*, *i*) and minimum Feret (i.e., the longest and shortest distances between two parallel tangents at the flower outline; dashed lines, Fig. 2*A*, *i*), and to quantify flower circularity from 0 to 1, where 1 is a perfect circle. We measured each flower's reflected light using an Ocean Optics Jaz-200 spectrophotometer with the optic fiber tip (25-cm, 600-μm Premium Fiber, UV/VIS) at an ∼45° angle against the flower, using a 1-ms integration time, a boxcar of 10, and 10 averages. We standardized against the reflectance from a Spectralon diffuse reflectance white standard.

Abiotic Cues. We used a custom-built portable sensor to record humidity and CO_2 from within or close to single flowers directly at the time of observation. These were not compared with ambient conditions as values changed rapidly due to wind, sun, or other natural perturbations. The sensor was custom built by Daniel Veit, Max Planck Institute for Chemical Ecology, Jena, Germany, and incorporated the following components: CO_2 sensor, SENCO21002 (MB Systemtechnik); temperature and humidity sensor, SHT75, 667–5271 (RS Components); air pump, G12EB (Gardner Denver); 5 V (EXP-R24-347) and 24 V (68-066357) Voltage Regulators (Exp Tech Saarbrüken and ELV Electronik, respectively); and data acquisition, DAQ USB 6009 (National Instruments). The abiotic sensor was controlled with LabView (National Instruments) from a PC laptop. Measurements were made by placing a Teflon tube connected with the air pump within 1 cm of the flower to bring local air to the sensors. Sampling was performed for ∼3 min and averaged after removing the first 50 s.

Volatile Cues. Volatile signatures were collected using 5-mm-long, 1.5-mm i.d./3.5-mm OD polydimethylsiloxane (PDMS) tubes (Carl Roth Rotilabo, silicone tube) modified from a protocol by Kallenbach et al. (36). Tubes were prepared in 1:1 acetonitrile/methanol for 3 h, dried under nitrogen, and subsequently heated from 40 °C to 260 °C at 10 °C/min to 260 °C for 160 min under 4 bar nitrogen in a Tube Conditioner (Gerstel). PDMS tubes were then cooled to 25 °C, and the procedure was repeated. Conditioned PDMS tubes were stored in amber vials flushed with nitrogen at −20 °C until use. Volatiles were sampled for 4 h by three PDMS tubes suspended above the flower on a steel wire at up to 3 cm and covered with an ethanol-cleaned plastic cup to protect from sunlight and wind. Samples were stored in amber vials at 4 °C until analysis (36). Blank tubes were taken to each sampling site, stored with the samples, and analyzed along with the samples to identify potential contaminants (*SI Appendix*, Fig. S2*B*).

Desorption of volatiles employed a Gerstel Thermal Desorption Unit (TDU) in splitless mode and a Cooled Injection System (CIS 4) controlled by Gerstel Modular Analytical Systems Controller C506 and Gerstel Maestro 1 software. PDMS tubes were introduced into the TDU at 30 °C using a Gerstel MultiPurpose Sampler. After a 1-min delay at 30 °C, the TDU temperature was increased to 200 °C at 100 °C/min for 10 min. Desorbed volatiles were transferred at 210 °C and trapped in a silanized glass wool liner of the CIS at −50 °C using liquid nitrogen. After 0.20 min of equilibration, the CIS was ramped to 220 °C at 12 °C/s and held for 5 min. Volatiles were separated and identified in solvent vent mode with a purge flow to split vent of 30 mL/min at 1.5 min and vent flow of 70 mL/min; vent pressure at 7.07 psi for 0.01 min using an Agilent 7890B gas chromatograph coupled with a 5977A MSD mass spectrometer using an HP-5 MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with helium carrier gas at 1 mL/min. The column oven was kept at 40 °C for 1 min, increased to 180 °C at 5 °C/min with a 5-min hold, and finally increased to 270 °C at 25 °C/min.

Compounds were identified by MS in electron impact mode with ionization energy of 70 eV, a transfer temperature of 250 °C, and source and quadrupole temperatures of 230 °C and 150 °C, respectively. GC-MS acquisition was performed using Agilent MassHunter Workstation software B.07.02.1938. Qualitative analysis employed Masshunter Qualitative Analysis, version B.07.00, by matching the mass spectral data of the peak with library spectra (National Institute of Standards and Technology and libraries created from standards), comparing their relative retention index (C_6–C_{30} hydrocarbons; Sigma Aldrich), comparing their elution order, and comparing their retention time with standards. A silicon derivative peak, octamethylcyclotetrasiloxane (RRI-991, Basepeak *m/z* 281), was used as an internal standard, and relative ratios were determined based on normalized peak areas.

Statistical Analysis and Model Flowers. We performed two types of MVA for data sampled from each region individually, using Simca (MKS Data Analytics Solutions). We used PCA (*SI Appendix*, Figs. S4 and S5) to identify components that vary together and OPLS to separate variables associated with Hot or Cold flowers. The spectrophotometer data gave 1,500 data points for each flower. To avoid the color data swamping the dataset, we employed PCA on the color data alone and used the loadings from the significant components in the total data analysis.

Artificial lures were created using paper printed with an HP Laserjet Pro-500 Color MFP printer (M570dw; HP Inc.) with color verified by spectrophotometer. Corolla shape was manually cut, and odor blends added to microcentrifuge tubes were placed in the center of each flower or in the ground beneath the lure. The negative control consisted of a large black circle (5-cm diameter) with no odor in the microcentrifuge tube. We created a unique positive control for each region, which closely mimicked the color, size, and odor of a real, generally attractive flower species. In Uppsala, we mimicked a *P. fruticosa* flower (Fig. 4*A*, *vii*), in Sikkim a *Ranunculus*, and in Bangalore, a *Cosmos* flower.

In the field, we placed the eight artificial lures equidistantly in two to three circles with a 90-cm diameter (distance was set to control for potential crossover effects of volatile cues from nearby lures and confirmed by PDMS measurement in standardization trials). After placing the lures in the ground, we added the odor compounds. Volatiles were replaced after 2 h for longer observational periods. One of the circles was used to quantify the phenotypic and abiotic (not added, but measured for consistency) cues (*SI Appendix*, Fig. S8). To control for potential magnet effects (28) from our lures and surrounding flowers, as well as local abiotic and biotic factors such as microclimates and community effects, we executed two types of controls. First, all eight model placements were randomized across trials. Second, on several days multiple circles were created—one in a neutral area with no natural flowers and one in an attractive area where hoverflies were observed to visit nearby flowers (within 90 cm as in lure placement). In a few cases, we also assessed lure height as a potential factor for attractiveness. There was no significant difference between these perturbations, suggesting that local or community factors did not affect lure attractiveness. During 2–4 h, we quantified the number of hoverfly visits to each of the eight flowers in the circle (individual flies could not be scored, although obvious multiple visits to the same lure were not counted). Visits were identified as either a landing, if the hoverfly landed on the artificial lure, or as an approach, if a hoverfly performed a directed flight toward the flower (to within 5–10 cm; *SI Appendix*, Fig. S6 *A*, *C*, and *E*). As a comparison, we quantified visits by other insects (*SI Appendix*, Fig. S6 *B*, *D*, and *F*).

To determine if the visits to the different lures were significantly different from chance (equal visits to all lures), we performed a χ^2 test with significance of $P < 0.05$. We also compared the visits to the flower lures with the visits to the positive and negative controls, respectively, using a Fisher's exact test with significance of $P < 0.05$.

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