Current Biology

Supplemental Information

Mosquitoes Use Vision to Associate Odor Plumes with Thermal Targets

Floris van Breugel, Jeff Riffell, Adrienne Fairhall, and Michael H. Dickinson

Figure S1, related to Figure 1. CO_2 plume model and flight trajectories
(A) Photograph of the wind tunnel, showing the low-contrast checkerboard floor and the visual feature used in the experiments described in Fig 2.
(B) Comparison of measured CO_2 concentration as a function of distance from the source with our turbulent flow, particle diffusion model, which was used to calculate the olfactory experience for the mosquitoes trajectories.
(C) Distribution of the errors between the measured CO_2 concentration and the model.
(D-E) Example flight trajectories of female mosquitoes in the presence of a CO_2 plume. The colored arrowheads show synchronized points across side and top views as well as the odor trace. The spacing between the points (33 Hz intervals) indicates the animal's speed.

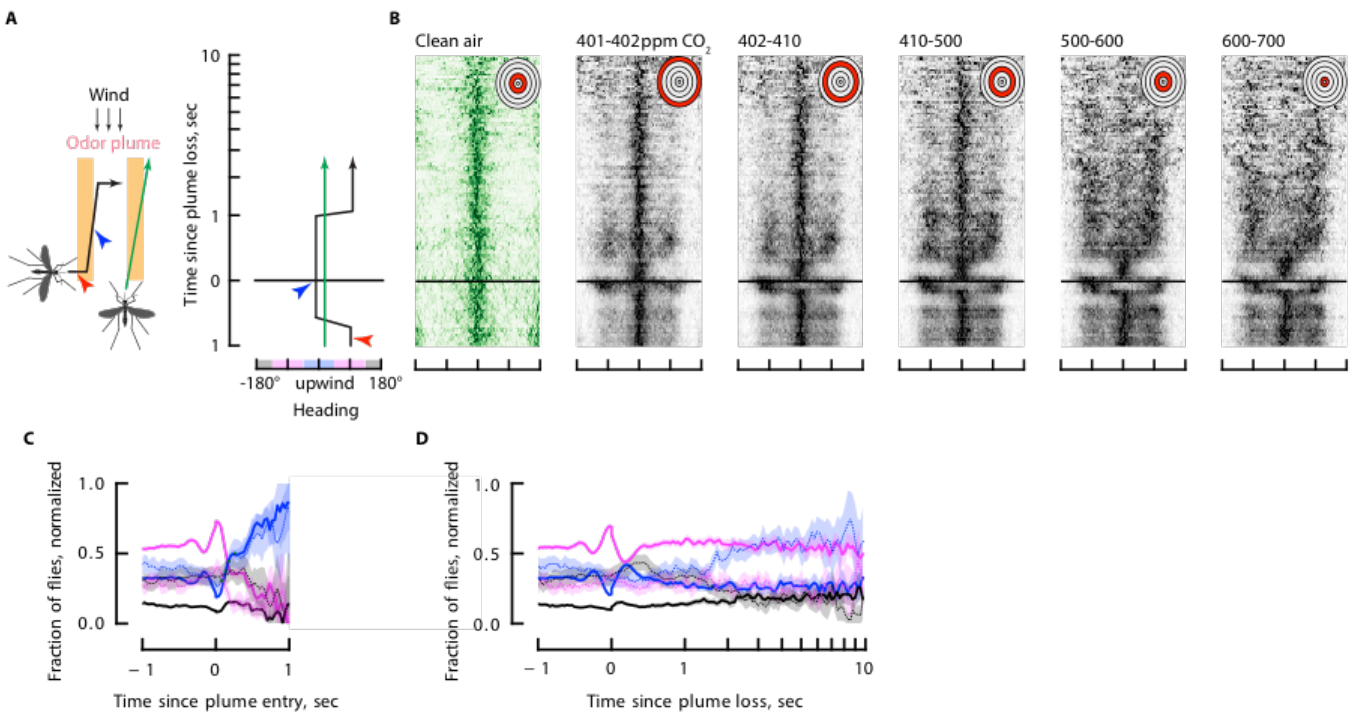

Figure S2, related to Figure 2. Mosquitoes surge and cast in response to CO_2 concentrations of 500 ppm.
(A) Cartoon of two flight trajectories and their respective representation on a plot of heading relative to the time when they exit the odor plume. This data representation is described in more detail elsewhere [S1]. The black trajectory depicts the stereotypical behavior we observed in the presence of a CO_2 plume; the green trajectory depicts the stereotypical behavior in the absence of any odors. The red and blue arrows indicate the point at which the trajectory enters and exits the plume, respectively.
(B) To graphically compile many trajectories, we plot the heading response of mosquitoes relative to the time when they exit the odor plume. These trajectory snippets are overlaid, and shown as a density map in which the colors are normalized such that each row contains a maximum and a minimum (higher color density indicates more trajectories). In the presence of CO_2, mosquitoes exhibit crosswind casts approximately 0.4 - 1 seconds after leaving the plume. Aligning the trajectories in this way requires that we define a behavioral threshold to CO_2. To determine at what concentration mosquitoes show a behavioral response, we selected trajectories that pass through five different annular regions of the plume and set the behavioral threshold to the minimum of that region. Based on these results, we chose the threshold of 500 ppm for subsequent analyses. Responses to a pseudo-plume of clean air did not show any clear changes based on our choice of threshold within these ranges (data not shown).
(C) To visualize behavior after entering the plume, we plot the fraction of trajectories flying upwind (blue), crosswind (magenta), or downwind (black) relative to plume entry for a pseudo-plume of clean air (dashed) and CO_2 (solid). Values were calculated by binning the normalized heading shown in b into four 90° sections corresponding to upwind, crosswind (left/right combined), and downwind (see color bars on the abscissa of b). Light colored shading indicates bootstrapped 95% confidence intervals.
(D) Same as c, with trajectories aligned to the point when the mosquitoes left the plume.

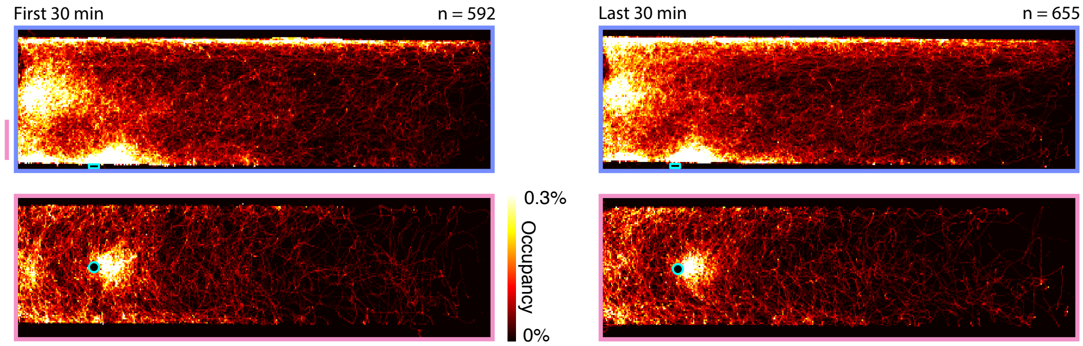

Figure S3, related to Figure 2. Mosquito's attraction to visual feature does not wane over time. The figure shows two heat maps similar to Figure 2a, for trajectories from the first and last 30 minutes of the 3-hour CO_2 presentation.

Supplemental Experimental procedures

CO2 plume calibration

In order to calculate the olfactory experience of each trajectory, we measured the CO_2 concentration inside the wind tunnel at 65 different locations with a LI-6262 CO2/H2O analyzer (LI-COR, Lincoln, NE). For each measurement, we positioned the LI-COR's input, and waited for the wind tunnel and CO_2 plume to reach steady state as indicated by the stability of the CO_2 concentration measured by the instrument (approximately 1 minute). The LI-COR was programmed to provide measurements at 10 Hz, for 1 second, at each of the locations. For points where the measured value was below or above 500 ppm, the average standard deviation was 7.95 and 28.0 ppm, respectively. We calibrated the LI-COR using a Scotty Transportable brand 400 ppm CO_2 in N_2 calibration mixture (Air Liquide America Specialty Gases LLC, Houston, TX), and found that the background concentration of CO_2 in our experimental chamber was within 2% of 400 ppm. To build a model of the plume, used the following equation that describes concentration (c) as a function position (x,y,z) according to particle diffusion in turbulent flow theory.

$$c(x,y,z) = \frac{Q\bar{u}}{2\pi\alpha_y\alpha_z u_*^2 x^2} \cdot exp - \left\{\frac{y^2\bar{u}^2}{2\alpha_y^2 u_*^2 x^2} + \frac{z^2\bar{u}^2}{2\alpha_z^2 u_*^2 x^2}\right\} + b,$$

The parameter Q is the number of particles released per second, $\bar{u}$ is the flow rate, α is the measure of dispersion, u_* is the shear velocity, and b is the background concentration. The background concentration and $\bar{u}$ were measured empirically. We used least squares to determine best-fit values for the remaining free parameters (Q, u_*, α_y, α_z, and the x position of the source). A comparison of the model and our measurements is shown in Figure S1.

Heated glass objects

Testing the role of vision and heat sensing in mosquitoes required objects for which the visual and thermal signatures could be independently controlled. For these experiments we used ITO (indium tin oxide) coated glass squares (MTI Corporation, Richmond, CA). This coating is conductive, and to achieve the desired resistance as well as uniform heating we etched a simple pattern on the surface with a laser engraver, see Figure 2A. To maintain a temperature of 37° C we used a CN7500 PID controller (Omega, Stamford, CT) to regulate the voltage across the glass. The controller operated on the output of a thermocouple attached to the surface of the glass. The visual contrast of the nearly transparent glass objects were increased by placing a small disk of infrared-pass filter (#87, Kodak, Rochester, NY) over the surface.

Trajectory analysis

In Figure 3, our analysis required that we score when trajectories approached one of two objects. We defined an 8x8x4 cm^3 volume (length x width x height) that was centered over the object in the crosswind direction, and shifted slightly downwind in the wind line direction (see Figure 3F). This volume was chosen as it captures the area of primary activity of the mosquitoes (as shown in Figure 2B).

To calculate the fractions of trajectories that approached either object, we randomly divided the trajectories into twenty groups. For each group, we calculated the fraction of trajectories that approached either object. From these twenty values, we calculated the mean and 95% confidence interval of the mean through random resampling of these twenty values 500 times. Statistically significant groups were estimated using Mann-WhitneyU test with Bonferoni correction at a p=0.01 level.

To calculate the preference index for the test object compared to the control object (as in Figure 3E), we first calculated a preference index for each trajectory that approached at least one of the objects. We defined the preference index as the amount of time a trajectory spent in the test volume near the test object minus the time spent in the volume near the control object, divided by their sum. Approximately 25% of the trajectories approached both objects, except in the experiments in which a wet KimWipe was placed on the warm object, in which case 50% approached both objects. From these preference indices, we calculated the global mean, and bootstrapped the 95% confidence interval of the mean through random resampling of the individual trajectories 500 times. Statistically significant groups were estimated using Mann-Whitney u-test with Bonferoni correction at a p=0.01 level.

This simple approach of calculating preference index, however, ignores the complexity of the 3-dimensional trajectories. To take full advantage of the richness of our dataset, we next calculated preference index in a new way. For each trajectory, we selected the segments corresponding to the two seconds prior to when it entered either of the test volumes shown in Figure 3F, in addition to the segments where the mosquito was inside either of these volumes. Using these fragments, we calculated the preference index for the trajectory at different regions in space as the amount of time the mosquito spent in a particular 2x2 cm^2 crosswind region on the side of the wind tunnel closest to the test object compared to the control object. Thus, each trajectory produced a sparse spatial array of preference indices. We then calculated the mean array across all trajectories, and bootstrapped the 95% confidence interval for the mean of each region by randomly resampling the trajectories 500 times. Since many regions (in particular those far away from the objects) were only visited by very few trajectories, or none at all, we only show the mean preference index for regions where the 95% confidence interval was smaller than 0.5. This requirement implies that regions where the absolute value of the preference index is larger than 0.25 have a greater than 95% probability of indicating a region where the mosquitoes showed a preference index significantly smaller, or larger, than zero.

References

S1. Van Breugel, F., and Dickinson, M. H. (2014). Plume-tracking behavior of flying Drosophila emerges from a set of distinct sensory-motor reflexes. Curr. Biol. *24*, 274–86.