Title:

The complex swarming dynamics of malaria mosquitoes emerges from simple minimally-interactive behavioral rules

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Abstract

Complex coordinated group behaviors such as bird flocking and fish schooling often rely on intricate interactions among individuals. In these groups, neighbors exhibit strong mutual attraction, alignment, and collision avoidance. Here, we tested whether insect swarms arise from similar simple behavioral rules. Using high-speed videography and agent-based modeling, we identified the behavioral rules governing mating swarms of flying malaria mosquitoes, marking the first such analysis for any insect species. We found that, unlike birds and fish, mosquito swarming behavior is driven primarily by environmental cues like the sunset horizon and ground objects. In fact, interactions between conspecifics are limited to only close-range collision avoidance behaviors. Thus, insect swarming seems fundamentally distinct from bird flocking and fish schooling.

Main Text:

Few behaviors are as mesmerizing as the coordinated movements of flocking, schooling or swarming animals. Such collective behaviors can serve the purpose of foraging, mating, migrating, or protecting against predators (*1–5*). Studying swarms offers insights into social learning, information transfer and animals ecology (*6*, *7*). One key discovery about these apparently complex behaviors is that they seem to emerge from simple behavioral rules (*8*, *9*). The "boids" model suggests that animals follow three rules based on distance: attraction to distant individuals, alignment with nearby neighbors, and repulsion to avoid collisions. Our understanding of birds flocks and fish schools is largely based on these rules and models that integrate them (*10–13*), but it is still unclear if flying insects in swarms follow these rules and remains unknown how these swarming insects achieve coordination (*14–16*).

While birds and fish show high mutual alignment within groups (*17–20*), insect swarms appear comparatively much more chaotic (*14*, *21–23*). It has even been suggested that Diptera, the most studied order of swarming insect, do not interact within a swarm (*24*, *25*). However, more recent studies have shown various levels of flight path correlations in swarming insects (*16*, *23*, *26*, *27*). A quantitative model of insect swarming, such as the above-mentioned boids model, is needed to identify the behavioral rules driving these correlations in insect swarms.

Quantitative research on swarming in insects has focused on swarms of midges and mosquitoes (*21–23*, *28–33*), which swarm as part of their reproductive strategy. Males form swarms at dusk or dawn near visual landmark, so-called swarm marker, and females join the swarm to find a mate (*14*, *34–38*). While environmental cues like swarm markers and sunset are known to induce swarming, their role in shaping individual mosquito movements within swarms is still unexplored.

Swarming mosquitoes have been described to exhibit looping flight paths (*21*, *22*, *39*, *40*). This led to the modeling of mosquito swarming as damped harmonic oscillator coupled to the swarm centroid (*39*, *41*), and consequently to the claim that male mosquitoes follow the boids model behavioral rules (*15*). The proposed behavioral rules are based on the assumption that individual mosquitoes are attracted to the perceived center of the swarms, and that swarming mosquitoes align their flight direction with those of their neighbors. However, while there is quantitative evidences of collision avoidances between mosquitoes (*16*, *41*), mutual attraction and alignment remain unproven.

The argued attraction and alignment among swarming mosquitoes are based on observed correlations in their flight paths (*15*, *23*). However, these correlations might instead arise from simultaneous responses to environmental cues like swarm markers and sunset horizons (*21*). This is further supported by the importance of environmental cues for insect swarming (*38*), and the limited sensory capabilities of male mosquitoes, whose hearing is tuned to detect females rather than other males (*42*). Given that male mosquitoes likely cannot detect other males beyond a few centimeters (*16*, *43*), their capacity for mutual attraction or alignment is limited.

Here, we identified the behavioral rules governing malaria mosquito (*Anopheles coluzzii*) swarming and compared them to boids model rules (*8*), and suggested for swarming insects (*15*). We specifically investigated the extent to which swarming mosquitoes interact with each other and their environment.

Using a combination of videography-based experiments and agent-based modeling, we employed a three-step approach. 1) First, we used stereoscopic videography to track individual

swarming mosquito flight dynamics and to reconstruct the three-dimensional dynamics of the emergent swarm as a whole. 2) By combining these results with knowledge from literature (*16*, *26*, *30*, *33*), we identified the behavioral rules that might underly mosquito swarming. 3) We then developed and used an agent-based model to test how well the identified behavioral rules can reproduce the observed flight dynamics, both of individual swarming mosquitoes and of the emergent swarm.

Results

Experimental results

We conducted videography experiments with male *An. coluzzii* mosquitoes swarming above a black ground marker, and under simulated sunset conditions. We reconstructed 938 three-dimensional flight tracks of swarming male mosquitoes (Fig. 1A), revealing that swarms formed an upside-down elliptical cone above the marker (Fig. 1F) and published previous in (*30*). The major and minor axes of the elliptic swarm being oriented normal and parallel to the sunset horizon, and with swarm's density peaking at the center and thinning towards the edges (Fig. 1B,C). The swarm's three-dimensional shape can be modelled using three vision-based thresholds (*30*): a minimum and maximum of the marker viewing angle α ($\geq 24°$ and $\leq 55°$), and a maximum of the viewing angle deviation R_α ($\leq$ 4.5%) (Fig. 1F). Put together, these thresholds allow the delimitation of the three-dimensional space where swarming mosquitoes are most likely to be found.

Individual flight dynamics

Then, we quantified the underlying flight characteristics of the individual swarming mosquitoes (Fig. 2). Duration of individual flight tracks varied from less than a second, to a maximum of 232 seconds, and averaging 15 seconds. Swarming males exhibited stereotypic flight dynamics, as expressed by the temporal dynamics of their linear and angular flight speeds (typical example in Fig. 2A,B). They alternated rapidly between two flight phases: relatively straight flights paths and rapid sharp turns. These rapid turns are often referred to as saccadic maneuvers, and are typically exhibited by flying Diptera (*44*, *45*).

We identified when mosquitoes performed both straight flight and saccadic turns by detecting the local minima (valleys) and maxima (peaks) of angular speed, respectively (Fig. 2B). Swarming mosquitoes exhibited saccadic maneuvers on average every 0.66s±0.32s (mean±standard deviation). The resulting saccadic turn frequency of 1.5Hz is similar to the turn frequencies found in previous research on swarming mosquitoes (*21*, *39*). During a single saccadic turn, swarming mosquitoes changed their flight direction of 164.5° (mode), and so they almost turned completely in the opposite direction. These turn angles during swarming are much larger than the typical turn angles of saccadic maneuvers previously described in foraging flies and host-seeking mosquitoes (*44*, *46*).

During the flight sections between consecutive saccadic maneuvers, swarming mosquitoes were approximately flying straight, as the tortuosity of these flight trajectories was close to one (τ=0.84±0.14; Fig. 2D). We therefore define these flight sections between saccadic maneuvers from here-on as straight flight sections. The distance traveled by mosquitoes in these sections was on average 31cm±14cm, which is slightly less than the swarm marker size (of 40 cm) (Fig. 2E).These straight segments were primarily horizontal, directed towards or away from the sunset horizon (Fig. 2H and 2I, respectively). The climb angles γ during the straight flight sections were consistently close to zero (γ= 0° (mode); Fig. 2H). To analyze the directionality of the straight

flight sections we determined the mean flight direction of each section, as defined by the azimuth angle ψ. These flight directions combined show a bimodal distribution (Fig. 2I): straight flight sections that followed a saccadic maneuver distally from the sunset were oriented primarily towards the sunset ($y_{saccade}>0$: ψ=-91.8° (mode); Fig. 2I), whereas mosquitoes flew mostly away from the sunset after a saccade proximally to the sunset location ($y_{saccade}<0$: ψ=91.8° (mode); Fig. 2I).

Swarm dynamics

Next, we investigated how mosquito flight dynamics varied with their position in the swarm. In the swarm center, mosquitoes flew the fastest and accelerated the least (*30*) and Fig. S10*a-d*). In contrast, mosquitoes flew the slowest and accelerated most rapidly on the sides of the swarm, particularly in the regions adjacent to and distally from the sunset horizon ((*30*) and Fig. S10*a-d*). The spatial distribution within the swarm of angular speed is very similar to the distribution of flight accelerations, whereby angular speeds were lowest near the vertical central axis of swarm, and highest on the swarm sides, adjacent to and distally from the sunset horizon (Fig. 3A-D). When looking at azimuth angles between successive saccades, the swarm can be divided in two halves: a region proximal to the sunset horizon, and one distally from it (Fig. 3E-F). After a saccade located near the sunset, mosquitoes primarily flew away from the sunset, and opposite. Similarly, mosquitoes near the top of the swarm tend to fly downward after a saccade, whereas near the bottom of the swarm mosquitoes flew upwards after a saccade (Fig. 3G-H). All together, these results suggests that swarming mosquitoes, probably like midges (*28*), fly fast, horizontally and straight when crossing the swarm center, and perform rapid turns at the swarm boundaries.

Behavioral rules

These combined findings suggests that *An. coluzzii* males follow simple behavioral rules while swarming (Fig. 4). Swarming mosquitoes preferentially fly straight through the swarm center, where the marker viewing angle (α_{max}) is maximal, continuing until this angle reduces by 4.5%, triggering a turning maneuver (Fig. 1F) (*30*). Turns are sharp, horizontal saccades biased towards the swarm center, with vertical adjustments depending on the mosquito's position. After the turn, mosquitoes resume straight flight until the marker viewing angle decreases again, triggering another saccade. Finally, at close distances, mosquitoes avoid collisions (as found in (*16*)), by reversing their flight direction by 180°.

Modelling results

We developed an agent-based model to test how well these simple behavioral rules can reproduce the observed complex emergent swarm dynamic (see Material and Methods). The model accurately reproduced key aspects of mosquito swarm behavior, with simulated mosquitoes exhibiting swarm dynamics consistent with experimental observations (Figs. 5, S6-S10). Simulated mosquitoes primarily performed saccadic maneuvers at the swarm edges and flew mostly horizontally and straight through the swarm center, with flight directions oriented towards and away from the sunset (Figs. S8, S9). Still, these dynamics are directly resulting from the behavioral rules, and thus expected.

More striking is the fact that the model generated emergent properties not directly tied to specific rules. As in real swarms of midges and mosquitoes (*32*, *39*, *47*), the density of simulated mosquito was highest in the swarm center (Fig. 5A-D and S10*e-f*), and distributions of straight flight path distances and durations were similarly distributed to those observed experimentally

(Fig. S11). The model also replicated the radial acceleration field observed in real swarms, where mosquitoes accelerate towards the swarm center, with maximum acceleration at the edges and minimum at the center (Figs. 5E-H, and as in (*33*, *37*)). This field arises from saccadic turns at the swarm boundary followed by straight flights towards the vertical centerline.

Our experimental data revealed that neighboring mosquitoes accelerate away at distances lower than 1.4 cm, likely due to evasive maneuvers (*16*), but accelerate towards each other at larger distances. These positive accelerations maybe the by-product of simultaneous flight behaviors of conspecifics, not mutual attractions. We tested this in the model by comparing versions with and without a collision avoidance rule (Fig. 5L). Both models reproduced the positive acceleration towards neighbors at distances over 1.4 cm, suggesting that synchronized movements in swarms can emerge from reaction to environmental cues alone, without direct interactions between conspecifics (Fig. 5K, L). Still, only the model with collision avoidance captured the repulsive accelerations of real mosquitoes at close distances (Fig. 5K), showing that a minimal amount of interaction is needed to capture the whole swarming behavior of mosquitoes.

Discussion and conclusion

Our combined experimental and modelling results show that the complex emergent swarming of malaria mosquitoes can be modelled based on a small set of behavioral rules. Like in flocking bird and schooling fish models, these behavioral rules include attraction, alignment and repulsion rules. But strikingly, here the attraction and alignment behaviors are not towards conspecifics, but instead relative to external environmental cues: the swarming mosquitoes are attracted towards the center line above the visual ground marker, and they align their flight direction along a horizontal axis normal to the sunset horizon. Collision avoidance is with respect to conspecific, and occur only at very short distances of less than ~2.5 body lengths (*16*). In contrast, birds and fish display high level of direct interaction, at comparable distances for fishes (*12*, *18*) and larger distance for birds (*19*). This shows that insect swarming might be inherently different from natural flocking and schooling, and that the previously suggested apparent long-distance interactions between swarming mosquitoes are in fact not interactions, but instead the result of simultaneous responses to external environmental cues.

Notably, swarming dynamics in *An. coluzzii* were consistent regardless of their number (Fig. S4 and (*30*)), supporting previous findings that swarming behavior remains stereotypic regardless of swarm size as well as the idea that even a single mosquito can perform swarming behavior in the absence of any conspecifics (*21*, *36*). It is plausible that other insect species follow similar rules during swarming, though this requires further quantitative validation. For instance, *An. gambiae* mosquitoes have been observed to swarm near ground markers along an east-west axis (*29*), suggesting similar sunset-oriented behavior to *An. coluzzii*. In contrast, *Culex* mosquitoes display predominantly vertical movements during swarming (*21*), potentially influenced by different environmental cues. Our method could be applied to test these differences and explore how swarming behavior adapts to varying environmental conditions like wind or light perturbations (*37*, *39*, *48*), which may involve additional behavioral rules.

Our results do not address interactions between males and females within these mating swarms. Males are known to be strongly attracted to females entering the swarm (*22*, *35*), and it has been hypothesized that the high density of males at the swarm center allows them to quickly intercept females at the periphery (*32*). Understanding the behavioral rules governing female

interception is crucial for a complete understanding of dipteran mating swarms. The combined experimental and modeling approach developed here provides a foundation for investigating these interactions.

Given that mosquitoes and midges are vectors of diseases such as malaria and bluetongue (*49*, *50*), understanding their swarming behavior is crucial for developing innovative vector control strategies, including gene drive technologies (*51*, *52*) and the sterile insect technique (*35*, *53*, *54*).

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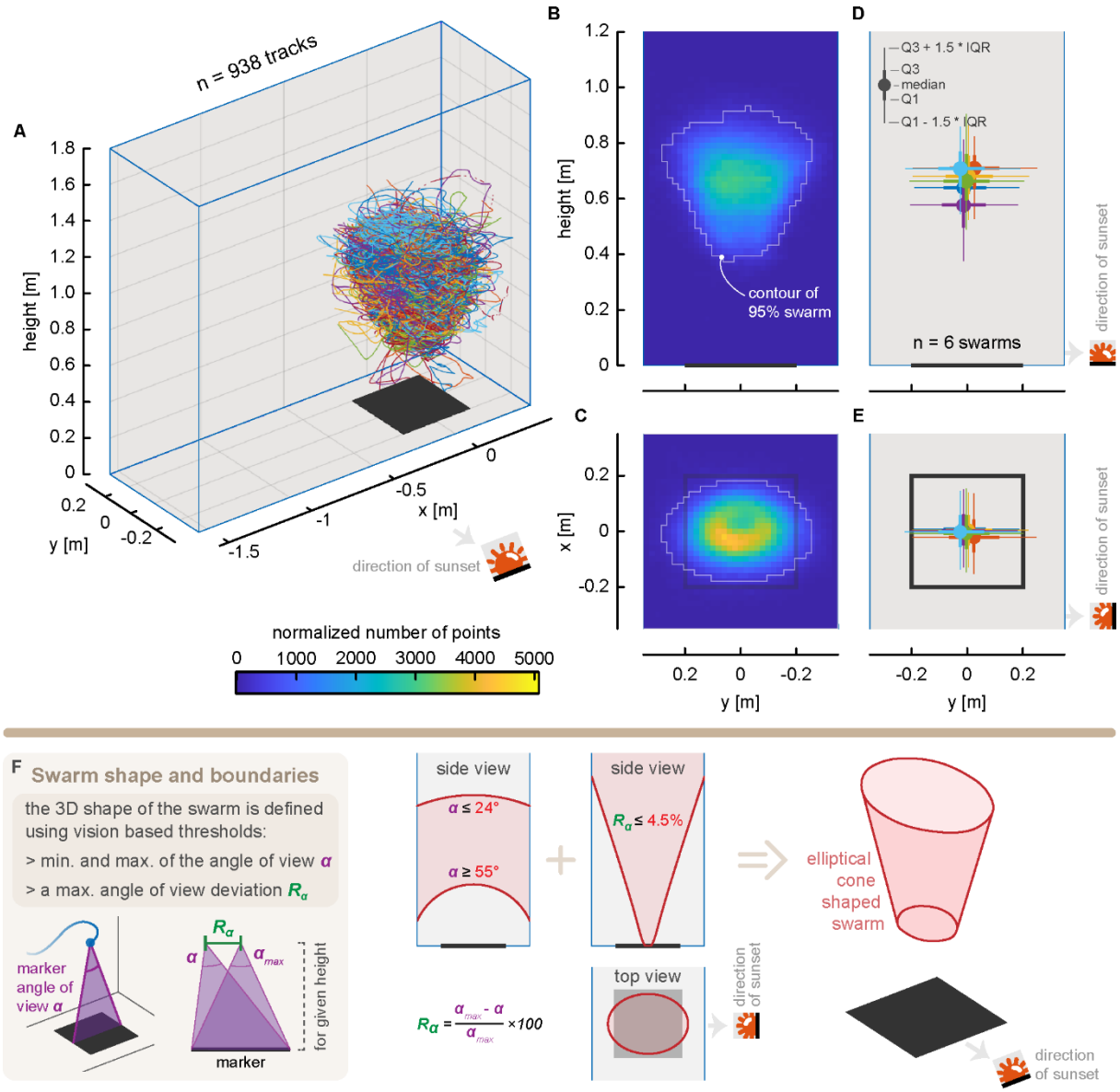

Fig. 1. 3D shape of mosquito swarm. (**A**) All recorded tracks of swarming male mosquitoes showed in 3d above the swarming marker (in black) and inside the flight arena. (**B-C**) Positional density of swarming mosquitoes presented as the total number of recorded position per cell (2x2cm) for all replicates (*i.e.* 6 swarms × 3 recordings = 18). The top and bottom panels show the swarm viewed from parallel to the sunset horizon and from the top, respectively (see sunset symbol). The contour of the swarm comprising of 95% of the data points is shown in white. The swarm marker is shown as a black line or square. (**D-E**) Side view and top view of the median location and quartiles of each of the 6 swarms. (**F**) Legend and schematics explaining how the three-dimensional shape of the swarm above the square marker is defined using three vision-based thresholds *(30)*.

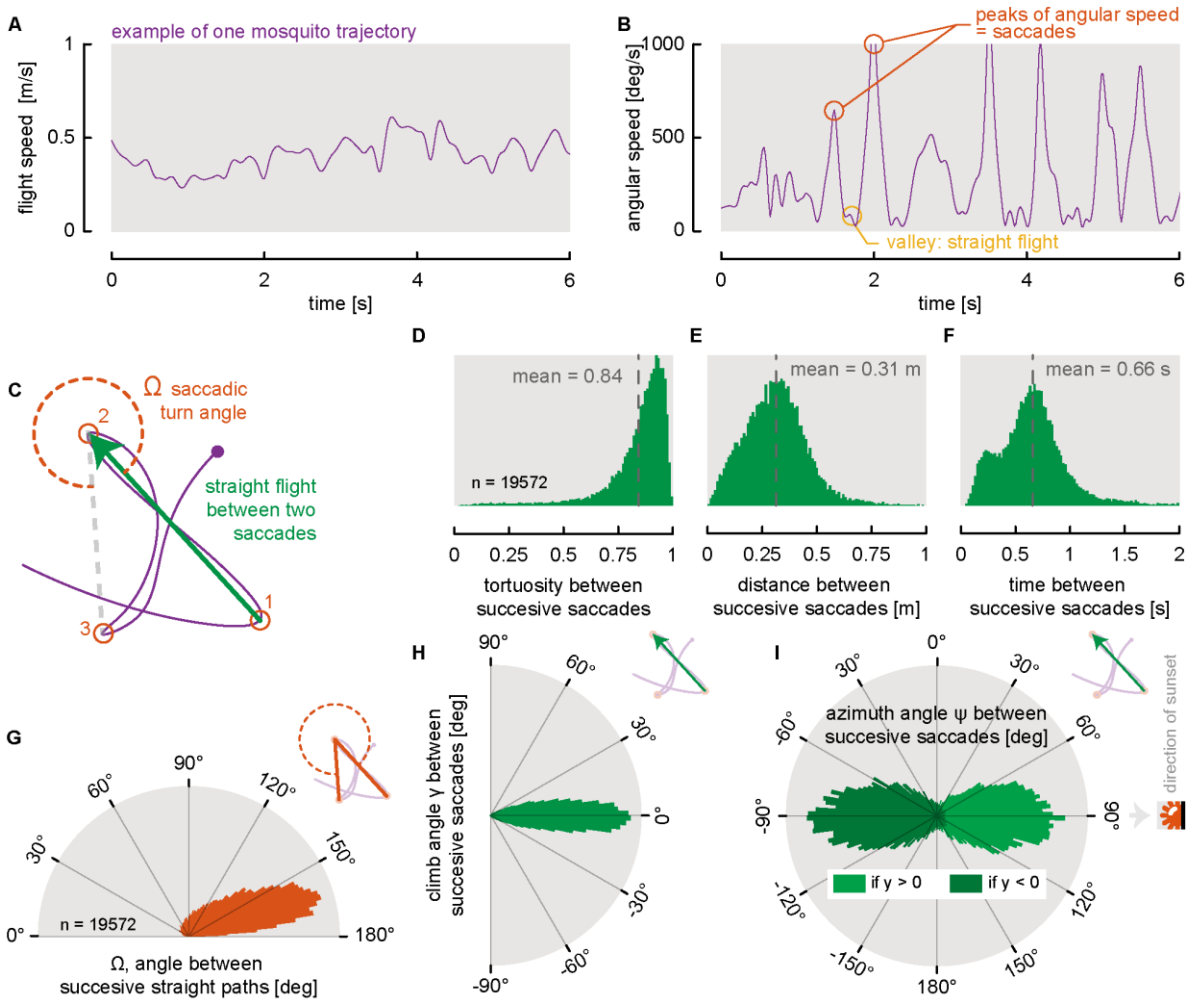

Fig. 2. Tracking results and saccade parameters. (**A,B**) Example of the temporal dynamic of the flight speed and angular speed of one swarming mosquito. These metrics are oscillating over time in a seemingly periodic way. Local maximums and minimums (i.e. peaks and valleys) of angular speed are identified to highlight the part of the track when the mosquito was flying straight (angular speeds close to zero), or exhibiting saccadic maneuvers (high angular speeds). (**C**) Schematics showing three consecutive peaks of angular speed and how the path between successive peaks is defined. Small orange circles indicate the successive saccade positions. The orange dotted circle shows the saccadic turn angle between two successive straight paths (grey dotted lines). (**D**,**E,F**) Histograms showing the tortuosity, distance traveled and time between successive peaks of angular speed for all mosquito tracks. (**G**) Polar histogram of the saccadic turn angle **Ω** between two successive straight paths. (**H,I**) Polar histograms showing the climb angle (i.e. angle from the horizontal plane) and azimuth angle (i.e. angle on horizontal plane) between successive peaks of angular speed. A flying azimuth angle between two successive peaks equal to 90° corresponds to a direction towards the sunset.

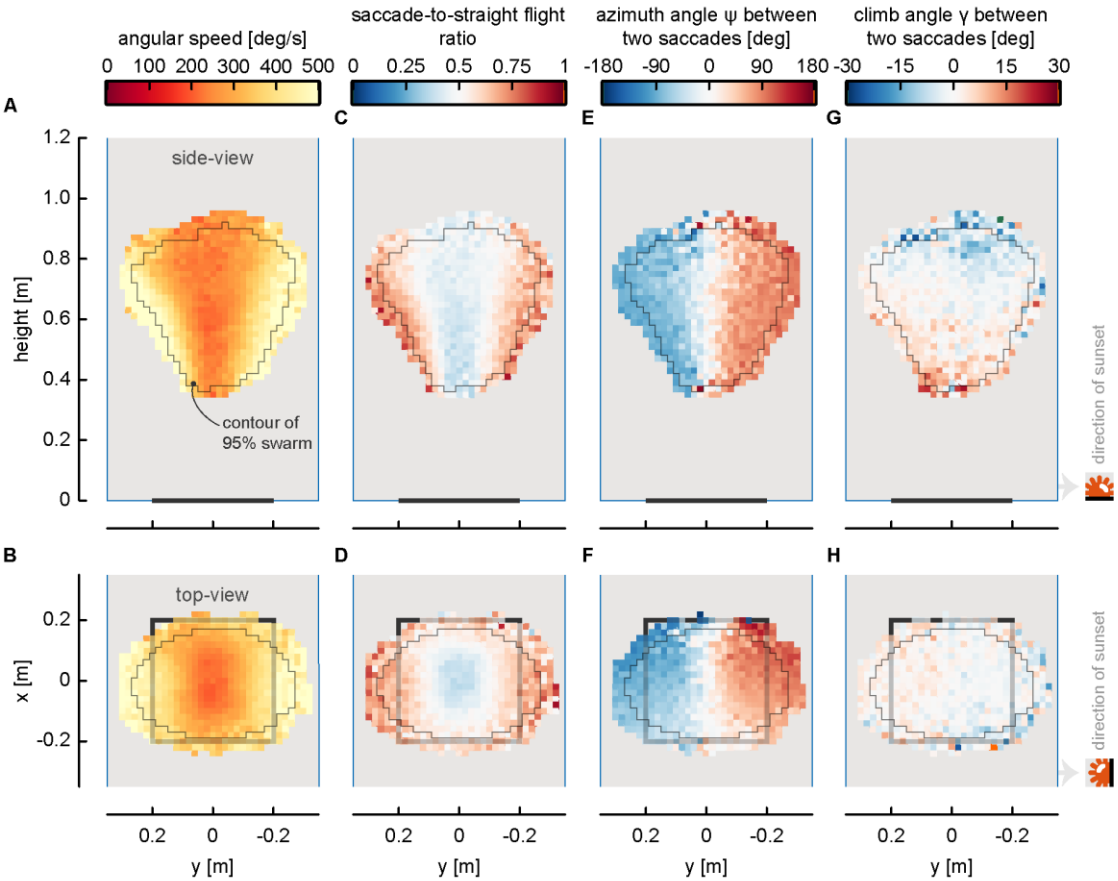

Fig. 3. Spatial characterization of saccades. (**A**, **B**) Spatial distribution of the mean angular speed viewed from the side or the top. The black bar and square represent the swarming marker. (**C-D**) Spatial distribution of the saccade-to-straight flight ratio, as quantified by the proportion of angular speed peaks and valleys. Regions in blue show areas where mosquitoes are flying straight on average (0 for 100% of angular speed valleys). Regions in red are where mosquitoes exhibited fast maneuvers the most often (1 is for 100% of angular speed peaks). (**E-F**, **G-H**) Spatial distribution of mosquitoes' azimuth and climb angles between two successive straight paths. Cells with less than an average of one track per recording period and per swarm (i.e. 3 recording periods * 6 swarm = 18) were filtered out. Cell size is 2x2cm for all panels. The thin grey line shows the contour of the volume comprising 95% of the three-dimensional tracking data.

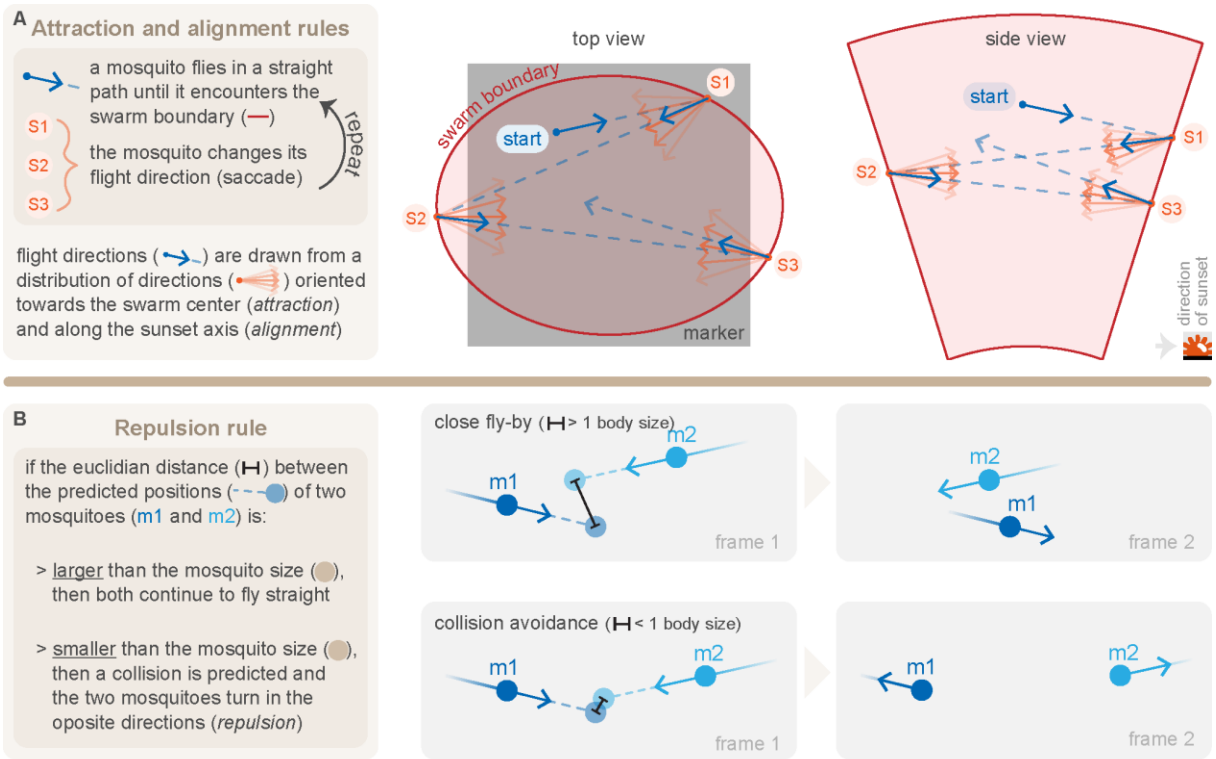

Fig. 4. Behavioral rules. (**A**) Legend and schematics describing the attraction and alignment behavioral rules of swarming male mosquitoes. Swarming mosquitoes alternate between (i) straight flight across the center of the swarm volume and (ii) saccades at the swarm boundaries. During each saccade, they redirect their flight towards the swarm center (attraction) and along the sunset axis (alignment). (**B**) Legend and schematics defining the repulsion rule used to prevent collision between close mosquitoes.

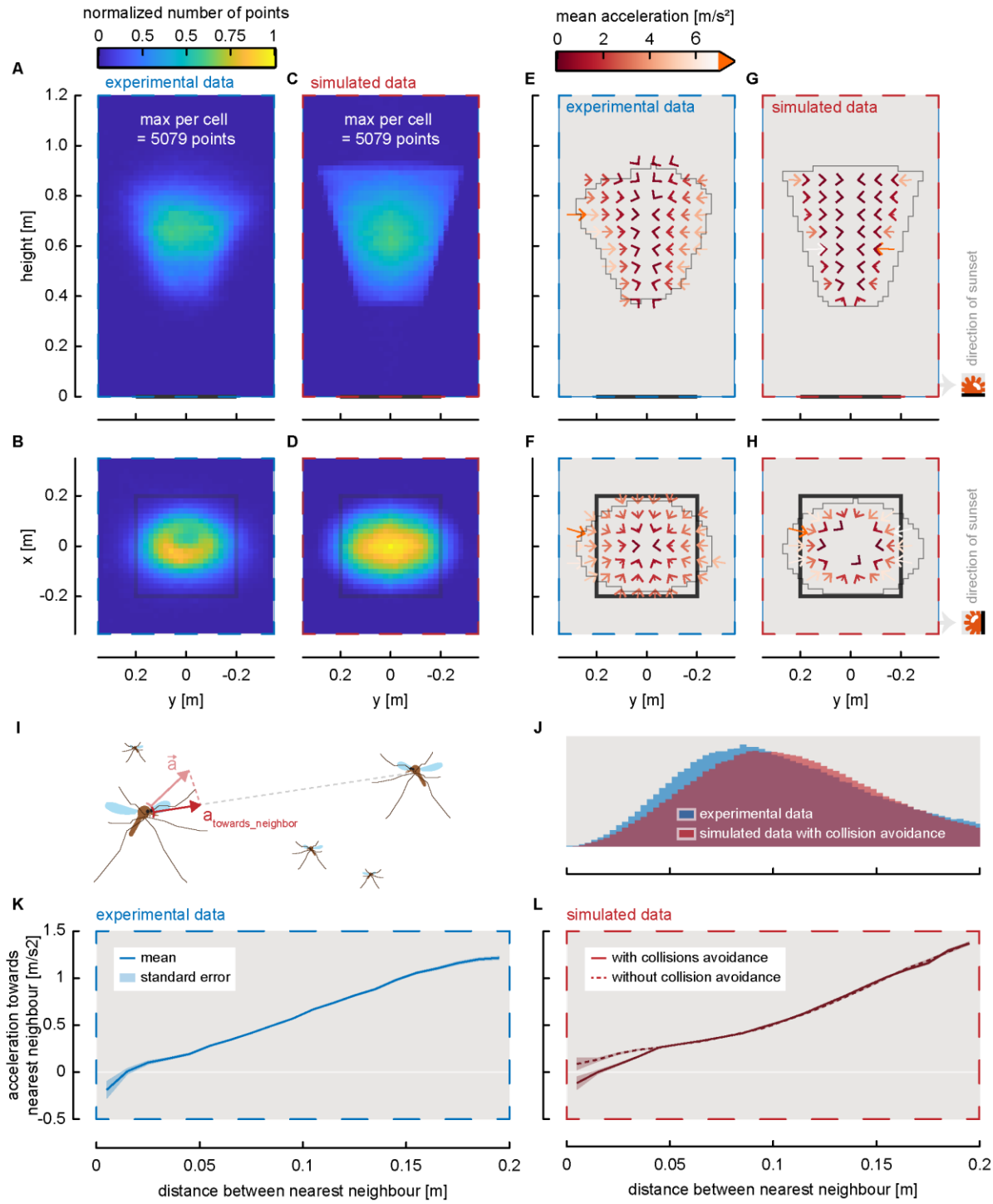

Fig. 5. Comparison of the spatial characteristics of real and simulated swarms. (**A-D**) Positional density of swarming mosquitoes from experimental and simulated data. The simulated data was generated using the behavioral rules described in figure 4. (**E-H**) Acceleration field from experimental and simulated swarms. Cells with less than an average of one track per recording period and per swarm (i.e. 3 recording periods * 6 swarms = 18) were filtered out. Cell size is 2x2cm for all panels. The thin grey line shows the contour of the volume comprising 95% of the three-dimensional tracking data. (**I**) Schematics showing how the acceleration towards neighbor is defined as the component of the acceleration vector of a mosquito directed towards its closets

neighbor. (**J**) Histogram of distances between nearest neighbors for the experimental and simulated data. (**K**-**L**) Graphs of the acceleration of individual mosquitoes towards their nearest neighbors versus the distance between the two neighbors for experimental data and simulated data using behavioral rules described in figure 4. When very close to each other's (< 1.4cm), mosquitoes on average accelerate away from one another, most likely to avoid collision (**K**). Integrating a simple collision avoidance mechanism in our simulation results in very similar results than in the experimental data (**L**). At larger distances between neighbors, mosquitoes are accelerating towards each other's (**K**). This is also true for the simulated data (**L**). This suggests that, excepted for collision avoidance, swarming mosquitoes do not exhibit noticeable interactions and that interactions are not necessary to create highly correlated swarming behavior.