Wings as impellers: honey bees co-opt flight system to induce nest ventilation and disperse pheromones

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ABSTRACT

Honey bees (*Apis mellifera*) are remarkable fliers that regularly carry heavy loads of nectar and pollen, supported by a flight system – the wings, thorax and flight muscles – that one might assume is optimized for aerial locomotion. However, honey bees also use this system to perform other crucial tasks that are unrelated to flight. When ventilating the nest, bees grip the surface of the comb or nest entrance and fan their wings to drive airflow through the nest, and a similar wing-fanning behavior is used to disperse volatile pheromones from the Nasonov gland. In order to understand how the physical demands of these impeller-like behaviors differ from those of flight, we quantified the flapping kinematics and compared the frequency, amplitude and stroke plane angle during these non-flight behaviors with values reported for hovering honey bees. We also used a particle-based flow visualization technique to determine the direction and speed of airflow generated by a bee performing Nasonov scenting behavior. We found that ventilatory fanning behavior is kinematically distinct from both flight and scenting behavior. Both impeller-like behaviors drive flow parallel to the surface to which the bees are clinging, at typical speeds of just under 1 m s^{-1}. We observed that the wings of fanning and scenting bees frequently contact the ground during the ventral stroke reversal, which may lead to wing wear. Finally, we observed that bees performing Nasonov scenting behavior sometimes display 'clap-and-fling' motions, in which the wings contact each other during the dorsal stroke reversal and fling apart at the start of the downstroke. We conclude that the wings and flight motor of honey bees comprise a multifunctional system, which may be subject to competing selective pressures because of its frequent use as both a propeller and an impeller.

INTRODUCTION

Insect wings have undergone approximately 285 million years of evolution as flight appendages (Dudley, 2000). The evolution of flight contributed to the dramatic diversification of insects in the Carboniferous period and has subsequently allowed winged insects to colonize nearly every terrestrial ecosystem (Dudley, 2000). Researchers often assume that flight performance is the only selective pressure driving the evolution of the flight apparatus. However, many winged insects have evolved secondary functions for wings. When at rest, insect wings can provide physical protection (e.g. forewings of beetles and grasshoppers; Tomoyasu

et al., 2009), help to absorb or dissipate heat (e.g. butterfly wings; Schmitz, 1994), provide camouflage, or signal to conspecifics and potential predators. The wings can also be moved for non-flight functions such as drawing air past the antennae to sense pheromones (Loudon and Koehl, 2000), producing sounds (e.g. stridulation), or for alternative forms of locomotion, such as skating across water surfaces (e.g. stoneflies; Marden et al., 2000). Although these alternative functions of wings are well known, few studies have examined how the physical demands of these non-flight functions differ from those of flight – a critical step in understanding how the various selective forces acting on the insect flight apparatus have contributed to its current form.

Here, we investigated the mechanics of a critical, non-flight use of the wings in social Hymenoptera – ventilatory fanning. Honey bees, bumblebees, paper wasps, yellow jackets, hornets and some stingless bees flap their wings while standing on the surface or at the entrance of their nests. This behavior presumably induces airflow through and around the nest that promotes convective cooling and/or gas exchange (Jones and Oldroyd, 2006). When nest temperatures or carbon dioxide concentrations rise above a threshold, honey bees assemble at the entrance of the nest and fan their wings, driving temperature and/or CO_2 values back below the threshold (Jones et al., 2004; Seeley, 1974) (Fig. 1). This behavior is critical to the success and fitness of the colony, as failure to regulate brood temperature can lead to cognitive deficiencies in adulthood (Jones et al., 2005). Considering that ventilation is crucial to healthy brood development, and that these insects invest so much time in a behavior that is likely to be energetically costly, we posit that there is a strong selective pressure on the flight system to accommodate this non-flight behavior.

Wing fanning also plays an important role in chemical communication among honey bees. During entry into a new nest site, honey bees broadcast volatile pheromones produced by the Nasonov gland at the tip of their abdomen. Bees that have located the entrance of the nest raise their abdomens and fan their wings, driving airflow across the Nasonov gland, which disperses pheromones that signal the location of the nest entrance to the rest of the swarm (Sladen, 1901; Beekman et al., 2006). This 'scenting' behavior is also used for other purposes, such as to direct disoriented workers back to the nest entrance after a nest disturbance (Ribands and Speirs, 1953).

Stationary wing fanning is a unique challenge for a winged insect. Fanning is used to impel (or blow) air along a solid, two-dimensional surface without displacing the insect's body, whereas the flapping motions associated with flight are used to propel the insect through an aerial, 3D world, generating air flows and forces that offset its weight and direct its motion. Insect wings have undergone several hundred million years of evolution as flight appendages that generate flow in a 3D, aerial environment. In contrast, ventilatory fanning in *Apis* presumably arose only after the appearance of eusociality in corbiculate bees, approximately ~87 mya (Cardinal and Danforth, 2011), and co-opting propulsive wings to serve as impellers presents some physical challenges.

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Fig. 1. A group of honey bees fanning at the entrance of a man-made hive. Note that fanning bees are oriented with their abdomens pointed away from the nest entrance, drawing air out of the hive.

Fig. 2. High-speed filming and kinematic measurement of fanning and scenting behavior. (A) Camera set-up used to film ventilatory fanning behavior. Two high-speed video cameras were focused on a point near the nest entrance and calibrated. When a bee began to fan, the entire hive was translated on a rail system so that the fanning bee was visible in the focal volume of the cameras. A similar set-up was used to film Nasonov scenting behavior. (B) Landmarks and kinematic parameters measured on fanning and scenting bees. The costal notch (CN) on the leading edge of the forewing, the wing hinges (WH) on both sides and the sting (ST) were digitized in each video frame. These landmarks were used to calculate abdomen angle (α), stroke amplitude (Φ) and stroke plane angle (β). Note that when the sting is positioned below the wing hinges (as in the illustration), abdomen angle is reported as negative. The photo used in this diagram was provided by Gord Campbell (CC BY-NC-SA 2.0).

First, the primary direction of fluid movement generated by the wings must be shifted from downward (as in flight), to horizontal (as in fanning). Second, the kinematics of flapping must be altered to avoid disadvantageous contact with the solid surface, which could cause wing damage (Mountcastle and Combes, 2014), reducing flight performance and survival (Cartar, 1992; Dukas and Dukas, 2011; Mountcastle et al., 2016). In this study, we explored three aspects of stationary wing fanning to better understand the selective pressures that this behavior may place on the flight system: (1) kinematic differences between wing fanning and flight, (2) the magnitude of air flow produced by an individual fanning bee, and (3) the potential role of wing–wing and wing–ground interactions in generating ventilatory flows.

MATERIALS AND METHODS
High-speed filming
All filming was performed on honey bees from domesticated, outdoor hives at the Concord Field Station (Bedford, MA, USA). Thirteen fanning bees performing ventilation behavior at the entrance of a beehive were filmed at 5000 frames s^{-1} using two Photron SA-3 cameras. The cameras were focused on a small focal volume (approximately 8×8×8 cm) at the nest entrance. The hive was placed on a 2-axis rail system so that when a fanning bee was identified, the hive could be re-positioned to center the fanning bee within the focal volume of the cameras (Fig. 2A). Before each round of filming, a 3D calibration was obtained by capturing a still photo of a calibration object with 14 markers with known spatial relationships. The calibration frames were analyzed using DLTdv5 MATLAB program (Hedrick et al., 2008). Because the calibration object was level, we were able to reference the horizontal plane in our video analysis. Filming of fanning behavior was conducted from 12:00 to 15:00 h on 20 September 2013. Bees were exposed to full sun during filming and the ambient temperature ranged from 23 to 26°C (recorded by the weather station at Hanscom Airforce Base).

A similar camera set-up was used to film 8 individuals performing Nasonov scenting behavior at 7500 frames s^{-1}. Approximately 500 workers from an artificial swarm of honey bees were placed at the entrance of an empty hive structure containing the swarm's queen. Upon locating the nest entrance, workers paused, lifted their abdomens and began to fan their wings, driving air across the exposed Nasonov gland. Filming of Nasonov scenting behavior was conducted on 14 August 2013 between 14:00 h and 17:00 h. Videos were captured in full sun and the ambient temperature ranged from 21 to 23°C (recorded by the weather station at Hanscom Airforce Base).
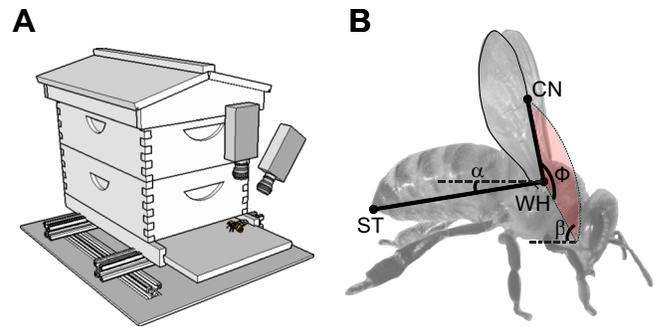
Quantification of kinematics
The costal notch on the leading edge of one wing, both wing hinges and the sting (tip of the abdomen) were digitized during five successive wing strokes using the DLTdv5 MATLAB program (Hedrick, 2008). The positions of these landmarks were used to calculate the stroke amplitude, frequency, stroke plane angle and abdomen orientation of fanning and scenting bees. Wing stroke parameters were quantified using methods described by Altshuler et al. (2005), so that our data could be directly compared with previously obtained measures for honey bee flight kinematics (Fig. 2B).

Stroke plane angle was determined by performing a regression on the path of the leading edge of the wing for five successive wing beats, and calculating the angle between this line and the horizontal plane. This metric describes the orientation of the plane through which the wing sweeps while flapping. Wing position was calculated as the instantaneous angle of the wing within the stroke plane, with the wing defined by a vector running from the wing hinge to the costal notch on the leading edge. Stroke amplitude was calculated as the difference between the maximum and minimum wing position angles, averaged over five wing strokes. Flapping frequency was determined by conducting a fast Fourier transform on the time series of wing positions over five strokes. The stroke-averaged angular velocity (rad s^{-1}) of the wing was calculated by dividing the stroke amplitude by the average duration of a half-stroke. Abdomen angle was calculated as the angle between the horizontal plane and a line connecting the midpoint between the wing hinges with the sting in the initial frame of the video (note that an analogous measurement during hovering flight was not available for comparison). Bees did not adjust their posture noticeably during the five strokes analyzed from each video.

Statistical analysis
For each kinematic parameter evaluated (flapping frequency, stroke amplitude, stroke plane angle and abdomen angle), we tested the null hypothesis that the median values of each flapping behavior

(fanning, scenting and hovering when available) were equal, using a Kruskal–Wallis test. In cases where the null hypothesis was rejected and data were available for all three behaviors, we conducted multiple comparisons among the behavioral modes using the Tukey method, to determine which behaviors exhibited differences in the kinematic parameter.

Qualitative flow visualization

We performed qualitative flow visualization of Nasonov scenting behavior, but were unable to use this technique for ventilatory fanning because of difficulties involved in inducing ventilation behavior in the laboratory. We visualized the flow generated by Nasonov scenting behavior in a transparent acrylic tunnel (5×5×50 cm) extending from a previously occupied hive, using a 532 nm sheet laser (Optotronics, Class IIIB, 2 W) and a high-speed camera (Photron SA-3) filming at 5000 frames s^{-1}. The hive box was seeded with suspended olive oil droplets, which diffused out of the box through the tunnel. The laser sheet was oriented downward toward the tunnel such that the laser sheet bisected the tunnel along the long axis. Particles moving along this plane were illuminated and visible to the camera.

Groups of 10 bees were placed in the tunnel. When the bees smelled the odor emanating from the nest box, they initiated Nasonov scenting behavior. Once wing fanning commenced, the hive/tunnel and the bees were re-positioned such that the laser illuminated a sagittal section of the air surrounding a fanning bee. An orthogonally positioned high-speed camera was used to film the motion of the particles.

RESULTS

Comparison of wing kinematics and body posture

Our comparison of wing kinematics during ventilatory fanning behavior, scenting behavior and hovering flight revealed that these behaviors involve kinematically distinct flapping modes (Table 1). Honey bees flapped their wings at significantly lower frequencies during ventilatory fanning behavior than during scenting behavior or hovering flight (P=0.0112 and 0.0007, respectively; Fig. 3A). In addition, stroke amplitude was significantly higher during fanning behavior than during hovering (P=0.0076; Fig. 3B). During scenting behavior, bees exhibited substantial variation in stroke amplitude (∼70–125 deg), but the median of the distribution did not significantly differ from that during ventilatory fanning or hovering (P=0.0835 and P=0.5207). The angular velocity of the wing during fanning, scenting and hovering was not significantly different. Both fanning and scenting bees flapped their wings through an anteriorly rotated stroke plane angle (>50 deg) relative to that of hovering bees

Table 1. Kinematic and body posture parameters of fanning, scenting and hovering bees

	Fanning (13)	Nasonov scenting (8)	Hovering (5)*
Frequency (Hz)	173.9±20.4	213.4±16.4	226.8±12.8
Amplitude (deg)	118.3±10.1	97.9±21.6	86.7±7.9
Stroke plane angle (deg)	54.5±8.6	52.2±9.8	−3.4±4.9
Abdomen angle (deg)	−1.9±7.3	15.8±12.0	–
Angular velocity (rad s^{-1})	721±122	731±178	685±57.5

All values reported are group means±s.d. Sample size for each behavioral group is indicated in parentheses and represents the number of individual bees from which the data were collected. *Note that values for hovering flight are reported from Vance et al. (2014) for comparison. The kinematic data for hovering individuals were originally collected by Altshuler et al. (2005), but were reported in more detail by Vance et al. (2014).

(P=0.0024 and 0.0138, respectively; Fig. 3C). However, despite maintaining a similar stroke plane angle, fanning and scenting bees exhibited significantly different body postures. On average, the abdomen angle of scenting bees was more than 15 deg higher than that of fanning bees (P=0.0018; Fig. 3D).

Flow visualization and velocity measurements

Our flow visualizations revealed that scenting honey bees draw air in from a broad area upstream, and accelerate it backwards in a narrow jet that runs approximately parallel with the surface to which the bees are clinging (Fig. 4A; Movie 1). A substantial portion of the upstream air appears to be drawn downward from above the bee. The flow visualization videos also revealed that scenting bees shed a vortex at the end of the upstroke. When viewed in the sagittal plane, this vortex flows along the dorsal surface of the abdomen and into the wake of the bee, persisting for approximately 1.5 body lengths. By digitizing the position of this vortex within the sagittal plane over 25 successive wing strokes of one bee (Fig. 4B), we calculated a mean (±s.d.) flow speed of 0.94±0.11 m s^{-1}. Because the vortex is traveling along the boundary between a low flow region (above) and the fast-moving current (below), we suspect that this is a slight underestimate of the flow speed generated by this bee during scenting.

Qualitative observations of wing–wing and wing–ground interactions

From the high-speed videos used for kinematic analysis and flow visualization, we made several unexpected observations of wing–wing and wing–ground interactions. In scenting bees, we observed wing–wing interactions during dorsal stroke reversal in some videos (Fig. 5A). In these cases, the leading edges of the forewings would contact each other at the end of the upstroke. The point of contact would then shift chordwise towards the trailing edge, as the leading edges began to pull apart at the start of the downstroke (see Movie 2). This wing–wing interaction is reminiscent of the clap-and-peel mechanism observed in many small insects that fly at low Reynolds numbers, which enhances lift (Miller and Peskin, 2009). In some of the videos, we did not see any physical contact between the wings; we cannot address whether this difference is due to inter-individual variation in behavior or to behavioral flexibility within an individual. We were also unable to determine whether wing–wing contact occurs during ventilatory fanning, because the contact was only visible with the use of a laser sheet during flow visualization. However, because the stroke plane angle is similar during the two behaviors, and stroke amplitude is often higher during fanning, we suspect that wing–wing interactions occur at least some of the time during ventilatory fanning as well.

In addition to wing–wing interactions during the dorsal stroke reversal, we also observed that the forewings commonly contact the ground during ventral stroke reversal, in both fanning and scenting bees (Fig. 5B). At the end of the downstroke, the wing first contacts the ground at the leading edge, and the point of contact shifts chordwise towards the trailing edge as the leading edge rises to begin the upstroke. Thus, bees appear to 'roll' the wings along the ground, from the leading edge to the trailing edge, which may help minimize damage caused by the collision.

DISCUSSION

Honey bees perform two non-flight, wing-flapping behaviors that are critical to the survival of the colony. Fanning behavior is used to ventilate the nest, in order to maintain a microclimate optimal for brood development (Seeley and Heinrich, 1981). Scenting behavior is used to drive air over the Nasonov gland and disperse pheromones that are key to communication in many contexts, such as
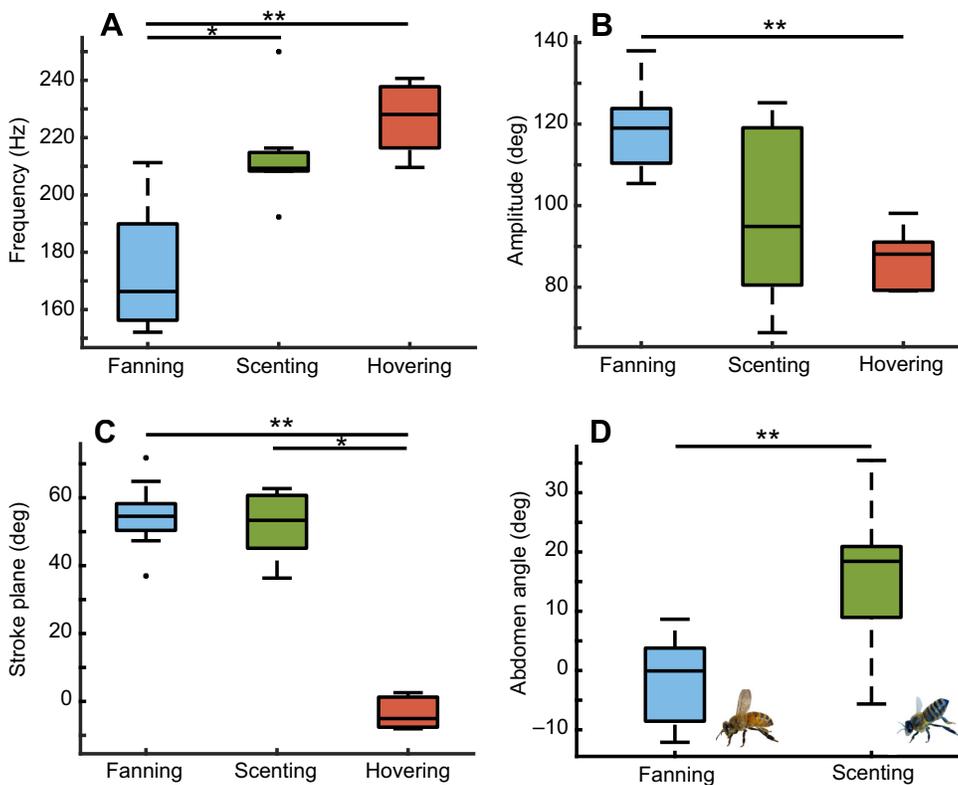
Fig. 3. Comparison of the kinematics of fanning, scenting and hovering. Flapping frequency (A), stroke amplitude (B), stroke plane angle (C) and abdomen angle (D), plotted according to the type of wing-flapping behavior (*$P<0.05$, **$P<0.01$). Values for hovering flight were calculated from Altshuler et al. (2005) and Vance et al. (2014); no corresponding measures of abdomen angle during hovering were available. Sample sizes are provided in Table 1 and indicate the number of individuals from which each parameter was measured.

coordinating the arrival of naive bees to a new nest site (Sladen, 1901; Beekman et al., 2006). In both behaviors, the wings are used as impellers, driving airflow past a stationary bee that is gripping a surface, rather than as propellers, generating downward flow to support a bee's weight in the air. This study demonstrates that the ventilatory fanning behavior is kinematically distinct from flight, and suggests that bees may employ unsteady flow mechanisms (e.g. clap-and-fling and wing–ground interactions) during these behaviors.

Wing kinematics

Neuhaus and Wohlgemuth (1960) filmed two fanning honey bees and measured their flapping frequencies (121 and 150 Hz), which were substantially lower than that of flying honey bees. They also measured amplitude (90 and 120 deg) for these bees, but they expressed lower confidence in these measurements because it was difficult to obtain video from appropriate angles and they were limited to 2D projections of the wing motion. Despite their limited sample size and methodological challenges, Neuhaus and

Wohlgemuth (1960) suspected that the kinematics of fanning and the kinematics of flight are different enough that they should be considered distinct behaviors. We were able to rigorously test this hypothesis with a larger sample size and with the use of 3D calibrations.

Indeed, one of the most striking kinematic differences we found between wing fanning behaviors and flight was in flapping frequency. Flapping frequency of ventilatory fanning behavior (mean±s.d. 173.9±20.4 Hz) was significantly lower than that reported for hovering flight (226.8±12.8 Hz). Scenting behavior had an intermediate mean flapping frequency (213.4±16.4 Hz), which was significantly higher than that during ventilatory fanning, but not significantly different from that during flight. The large differences we measured between the flapping frequencies of ventilatory fanning and hovering flight were surprising because the wings and flight motor of insects (especially those with asynchronous flight muscles, such as hymenopterans and dipterans) are classically described as a damped oscillator (Greenewalt, 1960). The lack of substantial variation in flapping frequency for many
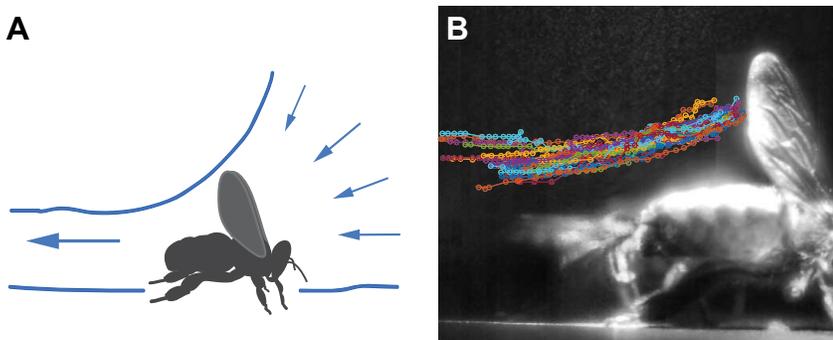

Fig. 4. Flow visualization. (A) A qualitative illustration of the flow generated by a scenting bee. This schematic diagram was drawn after observing flow visualization videos (see Movie 1). (B) Digitized positions of the location of a vortex shed at the end of the upstroke, as it moves downstream during Nasonov scenting behavior. Each colored line indicates the path of a single vortex during one wing stroke. Vortex paths during 25 successive wing strokes are plotted on the image. The vortex was visualized by illuminating particles suspended in the air with a laser sheet oriented along the sagittal plane of the scenting bee (see Movie 1). Note that the individual in this figure has an abdomen angle that is below average for scenting bees.
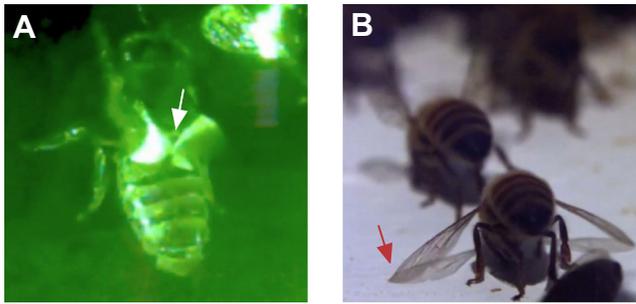
Fig. 5. Wing–wing and wing–ground interactions. (A) A chordwise transect of the wings of a scenting honey bee are illuminated during the dorsal stroke reversal by a green laser sheet. The white arrow indicates contact between the wings (see Movie 2). This clap-and-peel behavior is not observed during flight in honey bees. (B) A still frame from a high-speed video of ventilatory fanning behavior. The red arrow indicates contact between the wing and the ground.

species is consistent with this hypothesis (Greenewalt, 1960), and suggests that flapping frequency may be constrained to the natural frequency of the flight system (Ellington, 1999). However, our study reveals that honey bees performing ventilatory fanning routinely flap their wings at a frequency that is approximately 30% lower than during flight. Despite this reduction in frequency, fanning and scenting bees generate wing angular velocities that are similar to that of hovering flight. We also observed substantially more variation in flapping frequency during fanning behavior than was previously observed in flying honey bees (Altshuler et al., 2005). These observations suggest that bees may have more control over flapping frequency than previously thought.

The frequency of a damped oscillator system can be controlled by (1) changing the mass of the load or (2) changing the stiffness of the system. Honey bees are known to have a complex wing–thorax articulation and the transmission of force from the flight muscles and the wings is mediated by steering muscles and a series of sclerotized skeletal elements called pteralia (Nachtigall et al., 1998). Little is known about the role of this complex articulation in flight control in honey bees, but it is possible that bees could change the gearing ratio of the lever arm (i.e. the wing) by adjusting this articulation, changing the effective mass of the wing and ultimately the natural frequency of the flight system. In addition, the steering muscles could also be used to change the stiffness of the thorax–wing system in order to alter flapping frequency. This mechanism is widely accepted in Diptera, but it is typically thought to cause only small changes to flapping frequency (Ellington, 1999; Nachtigall and Wilson, 1967; Josephson, 1981). It is also possible that gripping the substrate during fanning behavior could contribute to changes in thorax stiffness or damping, which could lower flapping frequency. In general, variation in flapping frequency and the control of this variation has not yet been fully appreciated. Studying behaviors that exhibit broad variation in frequency, such as fanning and scenting behaviors in honey bees, could provide insight into how flapping frequency is modulated.

The reduced frequency during fanning behavior relative to hovering flight may also be related to differences in constraints on flapping frequency for these two behaviors. The aerodynamic constraints imposed by flight may be more significant in determining flight kinematics than those of the mechanical system. During flight, insects must generate enough lift to offset their body weight, whereas this constraint is removed when bees are performing flapping behaviors while holding on to a surface. It is possible that flight occurs at a narrow range of frequencies not as a result of control constraints (imposed by mechanical resonance at a natural frequency) but rather because of aerodynamic constraints (i.e. the need to generate significant amounts of force as effectively as possible). In the absence of these aerodynamic constraints, fanning and scenting bees may be free to tune their flapping frequency to meet other demands.

Our results on stroke amplitude lend some support to this idea. The distinctive high-frequency, low-amplitude wing stroke observed in flying honey bees (which is thought to maximize lift production via rotational mechanisms; Altshuler et al., 2005) shifts to a lower frequency, high-amplitude stroke in bees performing ventilatory fanning. This suggests that the flapping frequency and stroke amplitude observed in flying bees may optimize lift production via unsteady mechanisms, whereas the kinematics observed during fanning may be optimized for a different goal, such as maximizing the speed of air flow or the metabolic efficiency of flapping.

Wing–wing and wing–ground interactions

Many tiny insects, including white-flies and thrips, as well as larger insects, such as butterflies, use wing–wing contact at the dorsal stroke reversal to enhance lift (Weis-Fogh, 1973; Cooter and Baker, 1977; Ellington, 1984; Srygley and Thomas, 2002). During the end of the upstroke, the wings clap together, and at the start of the downstroke they fling (or peel) apart. As the wings peel apart, air is sucked across the top surface of the wings, helping to induce circulation around the wing (Miller and Peskin, 2009). This pattern of wing kinematics has not previously been described in honeybees during flight in natural conditions, but it has been shown to occur during flight in heliox, suggesting that honeybees may be able to employ this mechanism when high aerodynamic force is required (Vance et al., 2014). Our findings show that bees employ this kinematic device when performing Nasonov scenting (and likely also during ventilatory fanning) behavior.

One of the primary risks that might be associated with fanning and scenting behaviors is potential wing damage, as both behaviors require bees to flap their wings near the surface on which they are clinging. Given that high-frequency collisions with surfaces (e.g. vegetation) are known to cause wing wear (Wootton, 1992; Higginson and Gilbert, 2004; Foster and Cartar, 2011; Mountcastle and Combes, 2014) and wing wear leads to reduced flight performance and increased mortality (Cartar, 1992; Dukas and Dukas, 2011; Mountcastle et al., 2016), one might expect bees to reduce their stroke amplitude during these behaviors to avoid contact with the surface. However, we observed just the opposite; bees typically increase their stroke amplitude during fanning, and the wings often contact the ground at ventral stroke reversal, during both Nasonov scenting and ventilatory fanning behavior. The high-frequency collisions that occur between the wings and the surface are likely to cause considerable wing wear in honey bees, and possibly in other social hymenopterans that perform ventilatory fanning. In fact, we noticed that many of the individuals that we filmed had distal wing wear. One factor that may ameliorate this damage is the 'rolling' motion that we observed, as the wings contact the ground first at their leading edge and the point of contact rolls gradually towards the more fragile trailing edge. It is also possible that this rolling of the wing along the substrate generates additional flow by forcing a bolus of air (trapped between the wing and the substrate during supination) from the leading edge to the trailing edge as if by peristalsis. In addition, as the wing peels away from the substrate, suction would draw additional flow as the volume below the wing expands from the leading edge to the trailing edge.

Direction of impeller-induced flow and body posture

Our flow visualization results show that scenting honeybees draw in air from a broad area in front of and above their heads, and re-direct this air into a concentrated jet that flows parallel to the surface to which the bees are clinging (see Movie 1). The ground likely contributes to the resultant flow direction by deflecting flow induced by the bees. Because scenting and fanning bees have similar stroke plane angles, we expect that bees performing ventilatory fanning also generate downstream flow parallel to the surface. This directionality is important to the group-level ventilation behavior, because while flow generated by an individual bee may persist for only a few body lengths, bees fanning downstream can prevent the air from decelerating, and thus the group can achieve long-distance transport. If the flow were not parallel to the surface, this 'bucket brigade' of air flow would not be possible (see Movie 3).

Although the ultimate goals of bees performing ventilatory fanning and those performing Nasonov scenting behavior are different, we found that the stroke plane angle employed during these two behaviors was strikingly similar. One obvious indicator of scenting behavior is that the bees raise their abdomens to expose the Nasonov gland, which is located near the tip of the abdomen. Before conducting this study, we suspected that by raising the abdomen, scenting bees would achieve a more extreme stroke plane, allowing them to drive airflow upward, away from the surface to which they were clinging, which we thought might aid in broadcasting the pheromone. However, we found that while scenting bees do maintain a more elevated abdomen angle (mean±s.d. 15.8±12 deg) relative to fanning bees (−1.9±7.3 deg), they do not flap their wings through a significantly different stroke plane angle. This suggests that scenting bees may raise their abdomen during scenting not to alter the stroke plane angle but rather to expose the Nasonov gland to the fast-moving air induced by the wings. If the abdomen were held near the ground as in fanning behavior, the Nasonov gland would be located in the recirculation zone behind the body, and the concentration of pheromone in the air jet would likely be lower.

Conclusions

We have demonstrated that the flight system of honey bees performs several different functions, all of which are critical to the survival of the colony. Although the wings and thorax are often assumed to be specialized for flight, they routinely perform two additional tasks – ventilatory fanning and Nasonov scenting behavior – in which the flight system is used as an impeller. Ventilatory fanning behavior involves flapping frequencies and amplitudes that are significantly different from those employed during flight, and may subject the wings to irreparable damage. Use of the flight system as both a propeller and an impeller have likely imposed distinct (and possibly competing) selective pressures on it, which may have influenced its evolution and contributed to its current form.

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.149476.supplemental

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