Honeybees Prefer to Steer on a Smooth Wall With Tetrapod Gaits

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Abstract

Insects are well equipped in walking on complex three-dimensional terrain, allowing them to overcome obstacles or catch prey. However, the gait transition for insects steering on a wall remains unexplored. Here, we find that honeybees adopted a tetrapod gait to change direction when climbing a wall. On the contrary to the common tripod gait, honeybees propel their body forward by synchronously stepping with both middle legs and then both front legs. This process ensures the angle of the central axis of the honeybee to be consistent with the crawling direction. Interestingly, when running in an alternating tripod gait, the central axis of honeybee sways around the center of mass under alternating tripod gait to maintain stability. Experimental results show that tripod, tetrapod, and random gaits result in the amazing consensus harmony on the climbing speed and gait stability, whether climbing on a smooth wall or walking on smooth ground.

Key words: honeybee, tetrapod gaits, alternating tripod gait, climbing behavior, stability

Honeybee wagging on beautiful flowers is one of the most delightful images in summer. While honeybees’ fancy dance and superior flying capability are highly noticeable, their ability to climb a smooth, vertical wall is more intriguing because it might inspire robots climbing up walls. Although such ability has been well acknowledged, the gait characteristics of movement on a smooth wall have not been elucidated yet. Biomechanical approaches such as tarsal morphology, surface properties, and force measurements of honeybees and other phytophagous insects on a plant surface have been well studied over the past several decades (Evangelista et al. 2010, Reber et al. 2016, Bräuer et al. 2017). However, gait stability and transition of honeybees steering on a wall remain unclear.

Numerous studies have indicated that hexapod insects use tripod gait to maintain stability during climbing on the horizontal plane (Wilson 1966, Delcomyn 1971, Dickinson et al. 2000, Holmes et al. 2006, Ramdya et al. 2017). Stepping patterns of insects usually express rich diversity by the impressive flexibility and adaptability of their legs. During movement with a tripod gait, insects alter two triangles to support their bodies, and each support triangle consists of a front leg, a hind leg, and a contralateral middle leg (Wu et al. 2015). Also, three different flightless desert dung beetles utilize an additional gallop-like gait dragging with their hind legs while walking forwards to their burrow (Smolka et al. 2013). Insect locomotion usually requires highly coordinated working of the neuronal networks that control the climbing movements. Daun-Gruhn and Töth introduced a neuronal intersegmental network model of the stick insect walking system and studied the neuronal mechanisms underlying gait switches (Daun-Gruhn and Töth 2011). Barnett and Cymbalyuk described a model of insect locomotion to analyze the bifurcation control of gait transition in insect locomotion (Barnett and Cymbalyuk 2014). Furthermore, sensory feedback plays a critical role in the coordinated movements of insects. Couzin-Fuchs et al. (2015) examined the role of proprioceptive feedback in cockroach locomotion and established pymetrozine as a useful tool for further studies of insect locomotion. Ambe et al. (2018) proposed an analytical model to reveal the functional role of embodied sensorimotor interaction in hexapod gaits. In addition, Weihmann et al. (2017) investigated high-speed locomotion and gait changes of the cockroach (Nauphoeta cinerea), on two substrates of different slipperiness. The shift from the alternating tripod-odal to a metachronal gait pattern at high running speeds can help arthropods to avoid overstraining involved muscles and may facilitate energy efficient high-speed locomotion at the same time.

Several factors can affect the stability of locomotion. Kubow (1999) showed the potential stability importance of mechanical feedback in simplifying neural control. In later studies, Schmitt and Holmes (2000) showed that feedback was unnecessary by investigating the possibility of ‘passive’ stability in the context of the Lagrangian and Hamiltonian classical mechanics. Based on these descriptions, physical models, such as the spring-loaded inverted pendulum and the lateral leg spring, have been proposed to analyze the dynamic...
stability of legged locomotion (Schwind 1998). Subsequent studies suggested that running stability could be improved by the additional control of leg landing angle (Schmitt and Clark 2009, Blum et al. 2010). Shen and Seipel (2015) demonstrated that leg stiffness was also a significant factor that influences the stability of locomotion.

Compared with the lower-speed legged locomotion in the horizontal plane, few investigations have analyzed the stability of an insect crawling on a vertical surface. With the effect of gravity, maintaining stability is a challenge for most insects to crawl on a vertical surface, which is different from running on a wall or walking with a long stride. Currently, most legged robots usually utilize tripod gaits to move and make a turn (Tedeschi and Carbone 2014, Cully et al. 2015). The forward swaying motion of a robot body will lead to less stability. Here, an experimental platform was established to efficiently obtain the locomotive information of honeybees (Apis mellifera). The gait transition for honeybee climbing on the ground or on the wall was recorded, and we found a speed difference between the tripod and tetrapod gaits. We also observed the climbing behavior of honeybees and then compared and analyzed the proportion, climbing speed and stability of these gaits in the overall process in various climbing states.

Materials and Methods
Experimental Animals
The honeybees (Apis mellifera ligustica) were captured from the suburban area of Beijing and kept together in a beehive under an artificial light cycle, where the constant temperature was 25 ± 2°C and the constant humidity was 50%. We affirmed that no permissions were required for the experimental locations, and the field studies did not involve endangered or protected species.

Observation of the Crawling Process of the Honeybee
The experimental platform consisted of a detachable test glass box, a high-speed camera (Phantom, M110, Vision Research, Wayne, NJ) vertically fixed to a removable glass plate to record side-view images, and a computer to record these images. The high-speed camera was supported with a lifting platform and connected to a computer that set the frame frequency (800 fps), pixels (1280 × 800), and start and stop times. Two mirrors were placed at two flanks of the specimens such that each frame contained three views of the honeybee. We placed different colors on the three pairs of legs of the honeybee to keep clear track of the leg motions. The left legs were denoted as L1, L2, and L3, while the right side was denoted as R1, R2, and R3. In addition, the front of the head was considered as the reference point. We assumed that the near-side of the glass box in the vertical locomotion observation system represented the body plane, and the symmetrical plane of the body represented the sagittal plane. The pitch angle was the angle between the plumb line through the center of mass (COM) and the axis of the body (the line from the COM of the honeybee to the center of the head in a side view). The yaw angle was the angle between the moving direction and the axis of the body (the line from the COM of the honeybee to the center of the head in the front view). These key points on the image were extracted by a high-speed camera (1000 fps). Based on these coordinates, we calculated the information of locomotion parameters, such as the stride velocity, stride gait, motion trail, and continuous angle of legs. The crawling behavior of 30 honeybees was observed for climbing behavior both on a ground and a wall; all bees were from the same hive. Three stable movements were analyzed to eliminate interference induced by the fluctuation of the location parameters.

Results and Discussion
Honeybee Sways Its Body to Cooperate With the Tripod Gait to Climb Rapidly in a Straight Line
The periodic motion of honeybee legs is recorded during the fast-climbing phase. The results indicate the contribution of gait to locomotor speed. The legs of the honeybee, which are divided into two sets of stance legs (first set: L1, R2, L3; second set: R1, L2, R3), alternately support the forward body movement. That is, the front and rear legs on one side of the body move synchronously with the middle leg on the other side during a tripod ground locomotion. The synergy mechanism of these three legs is determined by analyzing a typical sequence to study the gait patterns of honeybees, using the head of the honeybee as the benchmark of a stride cycle. From state 1 to 3 (Fig. 1a and b), the honeybee moves from the former triangular area (linked by three touchpoints) between the substrate and legs (L1, R2, and L3) to the latter triangular area between the substrate and legs (R1, L2, and R3). From state 3 to 4, the legs, namely R2, L1, and L3, detach from the ground continuously. Finally, the honeybee moves into a steady state along with the leg L1 falling to the ground (state 5), completing a full revolution of tripod gait. Figure 1c illustrates the gait diagrams of the honeybee in a stride cycle. A tripod gait could start from the state in which the front left (L1), middle right (R2), and rear left (L3) legs are at a phase of stance while setting the remaining three legs to a phase of the swing.

The tripod gait has two power strokes per locomotor cycle. During each power stroke, three legs are on the surface (stance phase), whereas the other three legs are off the surface (swing phase). The resulting gait is characterized using a gait diagram that illustrates which legs are (stance) or are not (swing) in contact with the ground at each point in time (Fig. 1c). For a tripod gait of the honeybee, the contralateral middle leg touches the ground much earlier (approximately 12 μs) than the first and third legs, which provides the main grasping force to crawl on the vertical surface. The alternate tripod gait also keeps the body oscillating around the central axis.

The tripod locomotion of a honeybee depends not only on a series of gait sequences but also on how the honeybee body adjusts its behavior. The pitch angle was the angle between the longitudinal axis of the body and the vertical surface, and the yaw angle was the angle between the longitudinal axis of the body and the y-axis of the global coordinate system. Figure 1d and e show the measured pitch and yaw angles of the honeybee body and the body movement in the vertical and horizontal planes, respectively.

Two peaks are observed in the pitch angle curve. According to the gait diagram (Fig. 1a–c), the right midleg (R2) supports the honeybee body in the above 40% of the gait cycle, which corresponds to the first peak. During 40 to 80% of the gait cycle, the left midleg (L2) supports the body, which corresponds to the second peak. The magnitude of the pitch angle ranged from 15° to 19° (Fig. 1d).

Similarly, the yaw angle is generated by the swing motion of the body when the honeybee maintains its COM straightforward in an alternating tripod gait. Figure 1e illustrates that the yaw angle of the body almost changes in a sinusoidal rhythm. In other words, the body of honeybee swings around the crawling direction when the honeybee is crawling straightforward. During the initial stage, the legs R2, L1, and R3 touch the ground continuously. Meanwhile, the honeybee body starts to twirl leftwards while moving forwards. Then, the yaw angle increased to −9.51° at 13% of the gait cycle, when the body shows minimum twirling movement. After leg R2 leaves the ground, the honeybee body starts to twirl toward the right. At the same time, the yaw angle increases. The 40% gait cycle displaces a position between the two limits, where the yaw angle...
returns to zero. Maximum twirling of the body occurs at 62% of the gait cycle, where the yaw angle is 8.85°.

**Synchronized Forelegs Guide the Turning Direction of Honeybee Along With the Gait Transition**

Although the honeybee alters its gait to change from one direction to another, the central axis of the honeybee is always consistent with the crawling direction. This phenomenon has been described in other insects. On the contrary to the classic tripod gait, honeybees propel their body forwards by the synchronized movement of the middle legs and that of the hind legs. Figure 2a and b illustrate that the honeybee starts to crawl toward another direction at 40% of the gait cycle, where the angle between the turning and original directions was 28.84°. During the initial stage, the yaw angle increases to 9.35° at the 13% of the gait cycle, at which the honeybee body twirls to the maximum swing amplitude (Fig. 2c). Then, the honeybee body starts to twirl rightwards, further contributing to the decrease in the yaw angle. The yaw angle remains zero while the honeybee walked along the turning direction. This result indicates that the central axis of the honeybee remains consistent with the crawling direction. In addition, honeybees are perceived to depend on a distributed control mechanism, whereby the movement of each pair of legs depends on the phase of other segmental legs. For example, the hind leg movement depends on the current state of the middle legs.

**The Difference in Speed Is Found Between the Tripod and Tetrapod Gaits**

Figure 3a shows the local extreme values of instantaneous velocity curve. The green area represents the short period of tetrapod gait when the honeybee walks toward another direction. Maximum instantaneous velocity is measured at the end of an overlap phase when a new supporting triangle was taken into action. However, the mean stride velocity of the tetrapod gait is significantly lower than that of the tripod gait (Fig. 3b and c). This result suggests that the honeybee has adapted an alternating tripod for fast movement in a straight line and a tetrapod gait for turning movement.

When the honeybee crawls on a surface, unstable factors such as air distribution and surface forces are regarded as small
perturbations. The honeybee touches down when the COM was 3 mm away from the vertical surface. Stability starts to decline with increasing pitch angle. And even if the distance between the COM and vertical surface could reach 6 mm, the honeybee still crawls on the surface steadily. These results demonstrate the gait transition and movement stability of a honeybee crawling on a vertical surface.

Climbing Speed and Stability of Each Gait Are Compared and Analyzed in Various Climbing States

Honeybees usually use various kinds of gaits for climbing or ground locomotion. Here, we analyze the climbing behavior of 10 honeybees and show the proportion of these gaits in the overall process. Figure 4 shows that these gaits, which mainly included the tripod, tetrapod, and random gaits, show distinct duty ratio in different motion states. When climbing upward on a wall, the tripod gait consumed 69% of the total duration, which is the optimal choice for moving along a straight line. However, evident difference is found in the climbing speed and stability of these gaits between straight-line and turning movements. For turning, the tetrapod gait occupies 61.5% of the total duration, and become the most stable gait compared to the other gaits. In contrast, for climbing downward on a wall, the tripod gait is also the most stable gait for a straight-line movement (54.5% of the total duration), which is lower than that of climbing upward. Moreover, the duty ratio of tetrapod gait increases to 71.5%, which is higher than that of the other gaits, and is dynamically stable for turning.

Fig. 2. Tetrapod gait and yaw angle of honeybee body changing with time during steering. (a) Tetrapod gait for stride cycle of the honeybee. (b) Trajectory and gait switching of steering motion. (c) Variations in the yaw angle in a step cycle of tetrapod gait.

Fig. 3. Velocity difference of the center of mass between the tripod and tetrapod gaits. (a) The sequence of gait pattern and velocity. (b and c) Average moving speed measured in tripod gait (black bars) and tetrapod gait (white bars), showing the absolute (b) and relative (c) values toward the body length.
On the contrary to the optimal gaits for climbing on a wall, the tripod gait is the optimal gait for going straight on the ground, which occupies 55% of the total duration. At the same time, the random gait keeps the honeybee running at the fastest speed and takes up 39% of the total duration. For turning on the ground, the tetrapod gait ceases to be the best choice when the ground is smooth. Instead, the random gait is more popular or is the most needed method. The amazing consensus on the climbing speed and gait stability of these gaits, whether climbing on a smooth wall or walking on a smooth ground, is remarkably evident.

Conclusion
In this study, we conducted a series of experiments to uncover the gait transition for honeybees climbing on a wall or on a ground. Our experimental results showed that honeybees use the tetrapod gait rather than the tripod gait to change the direction of movement, both on a wall and on the ground. Although honeybees were known to use the tripod gait to walk, the precise connection between the tripod gait and swinging motion of honeybee body remained ambiguous. We found that honeybees adopted the tripod gait to provide the main grasping force to crawl on the vertical surface. The alternate tripod gait also kept the body oscillating around the central axis. Additionally, the honeybee swayed their bodies to cooperate with the tripod gait to climb rapidly in a straight line. More interestingly, honeybees adjusted nimbly to a tetrapod gait to change direction. Unlike the classic tripod gait, honeybees turned their bodies by the synchronized movement of middle legs and hind legs. Along with the gait transition, synchronized forelegs guided the turning direction of the honeybee. The central axis of the honeybee remained consistent with the crawling direction.

Furthermore, we analyzed the different behavior between climbing on the ground and on the wall by conducting comparative experiments. Our experimental results showed that the tripod gait was always the optimal choice for moving along a straight line, whether on the wall or on the ground. For turning, the tetrapod gait was the most stable gait compared with the other gaits. The tripod, tetrapod, and random gaits showed amazing consensus on the climbing speed and gait stability of the honeybee, whether climbing on the wall or walking on the ground.

This study contributes to perfect and enrich the system information of natural crawling strategies. Future work will focus on the optimal energetic cost of the locomotion of insects and the mechanism of the additional gait adapted for turning. In addition, we expect that the characteristics and mechanism of the gait transition of a honeybee can be applied to the development of a wall-climbing robot, especially in the design of its gait pattern.

Supplementary Data
Supplementary data are available at Journal of Insect Science online. https://mc.manuscriptcentral.com/LongRequest/js?DOWNLOAD=TRUE&PARMS=xik_22pnt5B7NcasyHPKXPKkossbVYm4NFDGUK42fSw8syy4RhEvWaiR8nhtg2Zd7i3bJKJwHz-t4WoBVs5fe9aPTNyAeKqmqnZihfB3Z9mPKvZ4Fzbd8xin-vKCrS9w3VBxCKjznEZuqgDWPewQ51KzTUrhopUaGdDQLhfcFMBUrQ1uatgVaqXRsevk2GofH71iPnBePA7dpxRFyp-d9w2nehps34J4DqinPbdVgmVsgigPY

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J.Z. and F.Z. collected the honeybee specimens and observed the gait switching phenomena. J.Z. and F.Z. carried out the measurement experiments of honeybee climbing behavior. S.Y. directed the project. J.Z., F.Z., and S.Y. wrote the manuscript. The authors have declared that no competing interests exist.

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