An experimental setup for decoupling optical invariants in honeybees' altitude control

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Abstract.
Bees outperform pilots in navigational tasks, despite having 100,000 times fewer neurons. It is commonly accepted in the literature that optic flow is a key parameter used by flying insects to control their altitude. The ambition of the present work was to design an innovative experimental setup that would make it possible to determine whether bees could rely simultaneously on several optical invariants, as pilots do. designed a flight tunnel to enable manipulation of an optical invariant, the Splay Angle Rate of Change (SARC) and the restriction of the Optical Speed Rate of Change (OSRC) in the optic flow. It allows us to determine if bees use the SARC to control their altitude and to identify the integration process combining these two optical invariants. Access to the OSRC can be restricted by using different textures. The SARC can be biased thanks to motorized rods. This device allows to record bees' trajectories in different visual configurations, including impoverished conditions and conditions containing contradictory information. The comparative analysis of the recorded trajectories provides first time evidence of SARC use in a ground-following task by a non-human animal. This new tunnel allows a precise experimental control of the visual environment in ecological experimental conditions. Therefore, it could pave the way for a new type of ecologically based studies examining the simultaneous use of several information sources for navigation by flying insects.

Keywords.
Optical invariant, insect flight, altitude control, ecological approach, invariant bias, invariants' removal, optical manipulation, motion vision.

Acronyms.
Optic Flow (OF), Optical Speed (OS), Optical Speed Rate of Change (OSRC), Splay Angle Rate of Change (SARC), Agent Environment System (AES), Virtual Reality (VR).

1. HIGHLIGHTS

- An experimental setup was designed to investigate whether the splay angle rate of change is implicated in bees' altitude control
- A flight tunnel was built making it possible to either bias or control access to optical invariants
- Splay angle rate of change is used by bees to control their altitude in a ground-following task

2. INTRODUCTION

Despite honeybees having 100,000 times fewer neurons than humans, they outperform the best trained acrobatic pilot in navigational abilities. Flying insects and pilots perform similar tasks such as take-off, cruise flight, and landing. These tasks are controlled visually despite widely differing visual systems (126 million photoreceptors per eye in humans *vs*. 48,375 photoreceptors per eye - 5,375 facets each one comprising 9 photoreceptors - in workers *Apis mellifera* [1]). Identifying the perceptual-motor principles underlying bees' navigation, and comparing it to that of humans would be useful in the design of flying aids and autopilots for drones in the near future.

To guide themselves in unfamiliar and cluttered environments and to control their altitude, most flying insects rely heavily on **Optic Flow** (OF) [2–14]. OF can be defined as a vector field of the apparent motion of objects, surfaces, and edges in a visual scene caused by the relative motion between an agent and the scene while being independent of the scene's texture [15–17]. It has been reported that different regions of the OF are used by insects to control their flight by maintaining constant a given level of optical speed [6]. Gibson [15] took these ideas one step further with his Ecological Approach to perception and action, through the concept of perceptual invariance. He demonstrated that, while any displacement of an agent gives rise to continuous changes in OF, some transformation properties remain unchanged.

These perceptual invariants (e.g., the focus of expansion, the rate of change in bearing angle, the relative rate of expansion of a surface, the splay angle rate of change) provide unequivocal information about the structure of the environment and more importantly for our purpose about the movement of the agent in relation to the environment (see review [10] for further details).

Two of the OF invariants that could be particularly relevant for an agent to control its altitude are **optical speed rate of change** - OSRC - (known to be used only by pilots [18] and flying insects [3, 6–9]) and **base line's splay angle rate of change** - SARC - (known to be used by pilots [18]). These two OF invariants offer the interesting possibility of providing information about altitude change without explicit measure of distance and speed of the agent.

The vast majority of previous studies have examined to what extent OSRC could account for altitude control in bees, by changing the orientation of the pattern stripes [19], or the pattern relative velocity [20], or by physically [7] or virtually changing the tunnel geometry [9], without examining the possible use of other perceptual invariants. As humans and bees can both use the same OF invariant (OSRC) [21], we can hypothesize that perceptual-motor coupling could be governed by universal laws or principles. There is a priori no reason to think that other invariants, like SARC, could not also participate in bees' altitude control. The experimental setup presented in this study will allow, for the first time, the determination of whether bees rely simultaneously on several OF invariants for altitude control, as was shown in humans for lateral positioning in a corridor [21].

To this end, we designed a flight tunnel allowing precise manipulation of the information content of the environment, such the two OF invariants could be either deleted or biased. This innovative dedicated flight tunnel will allow, not only a determination of whether bees also use SARC to control their altitude, but also to identify their integration process underlying the simultaneous use of these two OF invariants to control altitude. This method paper aims to detail this innovative device and to explain how this approach would allow a renewal of questions and guide future research towards new scientific challenges.

3. DESCRIPTION OF TWO ALTITUDE-RELEVANT INVARIANTS

Optical invariants give unequivocal access to the state of the agent-environment system (AES) [15]. In other words, for a specific task, once detected the invariant specifies the state of the system [22].

For a flying agent performing a ground-following task, OSRC and / or the SARC (described below) would unequivocally reveal a change in altitude. The innovative setup detailed in the following sections was designed to decouple these optical invariants and to simultaneously record the flight paths of the bees.

A. Optical Speed Rate of Change (OSRC)

When an agent is flying above the ground, objects constituting its visual environment sweeps its visual field. The optical speed of the ground is the ratio between the relative linear speed of the agent and its altitude above the ground (Fig. 1A.i). The closer the moving agent is to the ground, the faster the ground scrolls across its visual field (Fig. 1A.ii-iii). The optical speed of the ground (ω) can be calculated as follows :

$$\omega = \frac{\dot{x}}{z} \tag{1}$$

OSRC ($\dot{\omega}$) is the temporal derivative of the optical speed of the ground (ω), and can be calculated as follows:

$$\dot{\omega} = \frac{z\ddot{x} - \dot{x}\dot{z}}{z^2} \tag{2}$$

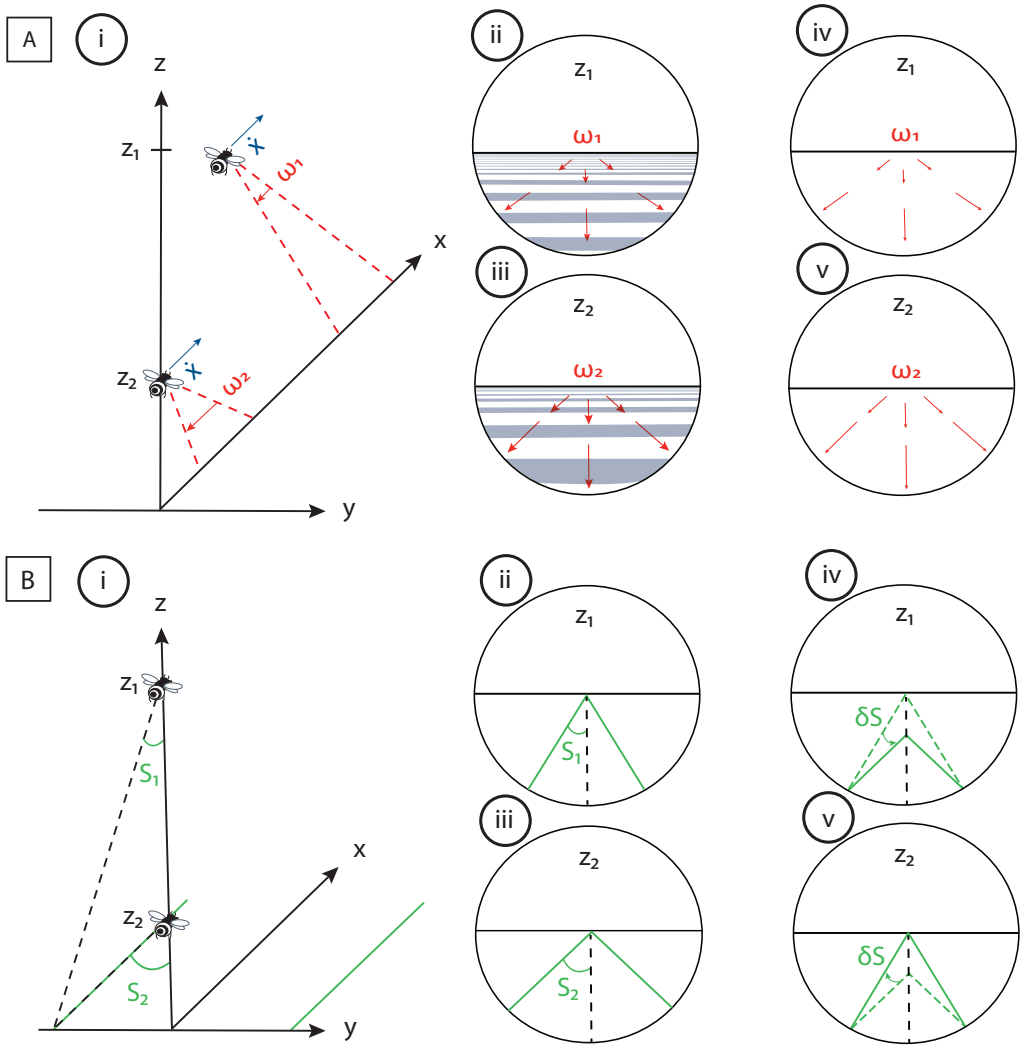

Fig. 1. Third person (**A.i** and **B.i**) and first person (**A.ii-iii-iv-v** and **B.ii-iii-iv-v**) representations of optical speed **(A)** and splay angle **(B)** in various experimental conditions. **A** - For a given forward velocity ($\dot{x}$) the optical speed (ω) is higher because the altitude is low (**A.i-ii-iii**). The optical speed is more easily accessible when the texture has more contrast (**A.ii-iii** *vs*. **A.iv-v**). **B** – The splay angle (S) can be defined as the angle subtended at the vanishing point by the direction of motion and a parallel line. The splay angle is also greater because the altitude is low (**B.i-ii-iii**). Our motorized rods make it possible to change the perceived altitude without changing the actual altitude. Converging rods give rise to an increase in the splay angle which species to a bee (when the laws of physics are not biased experimentally) that its altitude is decreasing (**B.iv**), while diverging rods give rise to a decrease in the splay angle which specifies an increase in altitude (**B.v**).

$\dot{x}$ is the observer's forward speed and z is the observer's altitude (Fig.1-A.i).

The optical configuration designed into our flight tunnel will allow us to uncouple the x-axis from the z-axis. It has been demonstrated that the bee's forward speed ($\dot{x}$) is controlled by the minimum section of a tunnel (here, the width) [6, 23], and using vertical stripes on lateral surfaces while manipulating the optical speed of the ground does not affect $\dot{x}$ [6]. Consequently, the bee's forward speed could be assumed to be quasi constant, then $\ddot{x} = 0$. We can therefore simplify Eq. (2) as follows:

$$\frac{\dot{\omega}}{\omega} = -\frac{\dot{z}}{z} \tag{3}$$

In our tunnel, the width does not vary so a bee goes through the tunnel at a constant speed. As a result, any change in the OSRC reveals a change in altitude.

B. Splay Angle Rate of Change (SARC)

When an agent flies over a flat surface covered with lines parallel to the direction of travel, these lines converge to a single vanishing point on the horizon. The splay angle was defined by Flach *et al.* [18] (Eq. 4) as the angle subtended at the vanishing point by the direction of motion and the parallel lines, as shown in Fig.1-B.i. A loss of altitude gives rise to an increase in the splay angle, whereas an altitude gain results in a decrease in the splay angle (Fig.1-B.ii-iii). Following Gibson's reasoning, the SARC is related unequivocally to a change in altitude and all the agent has to do to keep the same altitude is to negate any change in splay angle. The optical invariant lies in the SARC. Flach *et al.* [18] defined the SARC by means of Eq. 5.

$$S = arctan(\frac{y}{z}) \tag{4}$$

$$\dot{S} = (-\frac{\dot{z}}{z}) \times cosS \times sinS + (\frac{\dot{y}}{z}) \times cos^2 S \tag{5}$$

The SARC ($\dot{S}$) is the temporal derivative of the splay angle (S), y is the lateral position and $\dot{y}$ is the agent's lateral speed, z is the height above the ground and $\dot{z}$ is the agent's vertical speed (Fig.1-B.i). As bees remain quasi centered in the narrow tunnel, $\dot{y} = 0$:

$$\frac{2\dot{S}}{sin(2S)} = -\frac{\dot{z}}{z} \tag{6}$$

As a result of the design of our flight tunnel, any change in the SARC reveals a change in altitude.

4. MATERIALS

A. Flight tunnel

The flight tunnel is rectangular (220-cm long, 71-cm high and 25-cm wide), the four walls are textured with a printed pattern of red and white stripes oriented perpendicular to the crossing direction (Fig. 2-A and Fig. S1). On ceiling and floor, the pattern is printed on one side of a plastic sheet, the other side being homogeneously mat white. These reversible sheets allow us to provide or not a reference for optical speed. Moreover, when removed, the sheets reveal mirrors covering the floor and / or ceiling. The double-mirror configuration (i.e., when the mirrors are visible on both floor and the ceiling) deprives bees of any ventral or dorsal visual information (See [9] for details). Relative to an entrance-to-exit path, the left wall consists of two non-UV-cut plexiglas panels (PMMA, 2,5mm) holding red gelatin filter stripes (Lee 51 Filters HT019) between them (see Fig. S1). These transparent walls allow 80 percent of the ultraviolet light to pass through. Through this window, insect's trajectories can be conveniently video-recorded.

Three entrances (5-cm diameter doors) and three exits (5x5 cm) are placed at 14, 34 and 52 cm above the floor on entrance and exit sides, respectively. This will allow experimenters to manipulate the entry altitude if necessary. Behind each exit, a 10-cm side box permits the placement of a reward. Both entrances and exits can be manually manipulated by the experimenter from outside the tunnel. Recently, the tunnel's entry was equipped with a radiofrequency identification (RFID) system (Microsensys, Germany). In future experiments, it will allow the individual recognition of bees, and temporal monitoring of the evolution of their behaviour.

B. Pattern providing the optical speed

A unique pattern is printed on plastic sheets and stuck on right wall but removable on ceiling and floor (Fig. 2-A). The same pattern is reproduced with gelatine filter stripes on the left wall to allow video recording. It consists of red and white stripes oriented perpendicular to the crossing direction. As bees do not possess red-sensitive photoreceptors [24, 25], so they perceive red stripes as darker ones.

These red stripes of two different widths (1 cm and 3 cm) form a simple 10 cm-wide pattern, visible in Fig. 2-A. The angular subtends of the stripes ranged from 5.7° to 53° (1–10 cm wide pattern viewed from a distance of 10 cm, respectively) and from 0.5° to 5.3° (1–10 cm wide pattern viewed from 1 m, respectively). Between the red and white stripes, the Michelson contrast is 0.47 on sheets and 0.25 on the plexiglas (see [7] for details).

The OSRC can be non-continuously manipulated by using three different textures on the ground (Fig. 2-A): transversal red stripes to provide a regular OSRC, homogeneous white texture to provide a weak OSRC, or a pair of mirrors (one on the ground, one on the ceiling) to cancel the OSRC. The OSRC could be also continuously manipulated by equipping our flight tunnel with a suitable rotating pattern on the ground (see [20] for details).

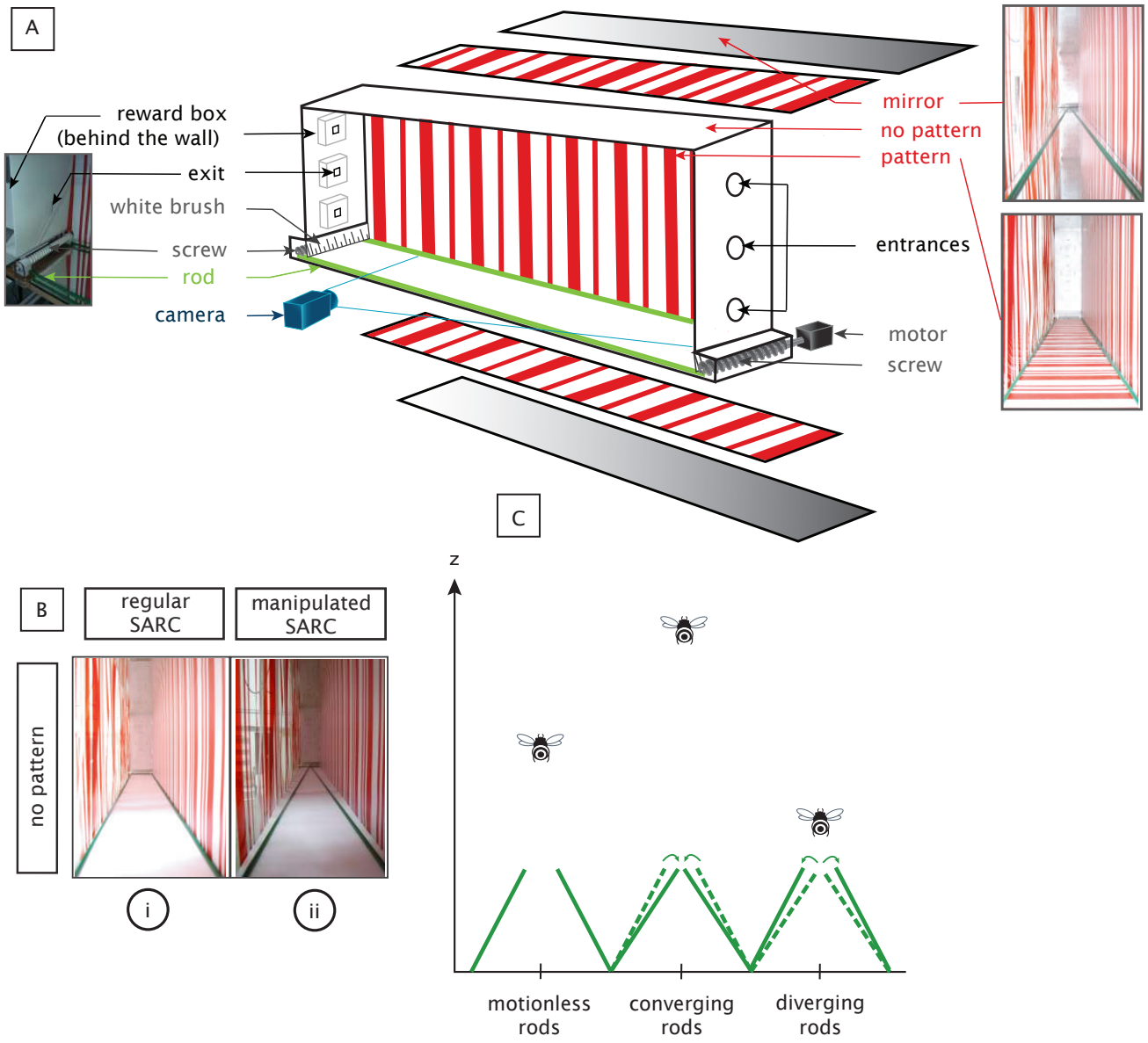

Fig. 2. Flight tunnel designed to manipulate OSRC and SARC **(A)**, optical invariants provided inside the tunnel **(B)** and predicted behaviour in bees **(C)**. **A** - Three textures can provide regular, weak or no OSRC on the ground. Two green rods linked to a two-way metric screw and moved by a motor allow us to manipulate SARC during the flight. Speed, acceleration, and amplitude of the SARC can be tuned. **B** - In (**B.i**) and (**B.ii**) a uniform blank sheet provides a weak OSRC. A striped pattern provides a regular OSRC. In (**B.i**), the splay angle is not manipulated, whereas in (**B.ii**) the rods are converging. The SARC is positive from (**B.i**) to (**B.ii**) and negative from (**B.ii**) to (**B.i**). **C** - Predictive altitude of bees induced by SARC manipulations inside the flight tunnel.

C. Rods providing the splay angle

The flight tunnel allows us to manipulate both the splay angle and its rate of change (Fig1-B.iv-v). Under the entrance and exit holes, just above the floor, a speed regulated DC motor operates a printed two-way metric screw. This screw mechanism is hidden by a white nylon strip brush to limit visual landmarks (Fig.2-A). On each side, the screw holds a green painted rod (8 mm in diameter) that runs through the whole tunnel length at each floor-wall junction. These rods are painted green because of the importance of green contrast in motion detection [24, 26]. Turning, the screw makes the rods converge (Fig.1-B.v) or diverge (Fig.1-B.iv), manipulating the SARC according to the experiments (Fig.2-B). Both the splay angle and SARC can be manipulated during an insect's flight to understand how they could be implicated in its altitude control.

The screw mechanism is motorized with a Crouzet motor: 80140043 – DC Motor 80 140 smi21 Series, 48 V, 94 W, 4000 RPM, 225 mNm. The metric screw, made in polylactic acid (PLA), is 10 cm long and its screw thread is 2 cm. The maximum usable motor speed is 4000 RPM linked to a gear ratio 1/200 to generate a SARC amplitude up to 120°/s. The rods' angular displacement is up to 2.8°; knowing that the bees' time-of-flight on average is ca. 2.4 seconds to cross the 1.6m-long recorded area, we could apply a step perturbation in SARC of any amplitude up to 120°/s. Moreover, this setup offers the possibility of tuning a latency into rod movement when a bee enters the tunnel, in order to record related altitude changes. In our experiments, we set the SARC ($\dot{S}$) amplitude perturbation at a unique angular speed of 14°/s.

D. Video recording and flight path analysis

The bees' trajectories were filmed at 100 frames per second with a high-resolution black-and-white CMOS camera (Teledyne Dalsa Genie HM640). A red filter is set in front of the camera monitoring bee's tracks. This process removes the red stripes on the trajectory records and optimizes the contrast between the bee and the background. The camera was placed sideways, 2.3 m from the left wall, and its field of view (160 cm in width, 71 cm in height) covered the whole height of the tunnel, from abscissa x = 0 cm to abscissa x = 160 cm in all experiments (Fig.2-A). Image sequences were recorded with StreamPix 7 software (NorPix, Inc., Canada), then calibrated, corrected, processed and analysed using a custom-made Matlab program (The MathWorks, Inc., USA), URL: https://github.com/rm1720/bees-applications. This program automatically determined the honeybees' flight height z in each frame as a function of the abscissa x or time t along the tunnel axis so that the bee's trajectory in the vertical plane could be plotted. 2D Coordinates of bees were sampled every 6 ms, then discretized through a binning (binMed, Matlab). Each bin represent the median of 25 coordinates, meaning 150 ms.

5. METHOD

A. Training and familiarization phases

Apis mellifera we are working with circulate freely in the Parc National des Calanques (Marseille, France). Two hives were set at 30 meters away from the experimental site. The bees were attracted on this experimental site by alimentary lures made of pure honey in plates placed meter by meter away from the hive.

Groups of bees were trained to fly along the tunnel set outdoors in a particular visual configuration. The training phase consisted of gradually moving a food reward (sugar, honey and water) from the tunnel's entrance towards its exit. After this training phase, bees traversed the tunnel following a single path to collect their rewards from a box behind the exit wall.

To allow the bee to be familiarized with the visual environment provided by the setup, a 1 hour familiarization phase preceded each experiment. During this phase, bee trajectories were recorded in a visual configuration allowing the perception of both invariants (OSRC & SARC). This way, we ensured every recorded bee had already gone through the tunnel 10 times. To choose the time of familiarization phase, 10 bees have been marked with gouache paint on the top of their abdomen to be individually recognizable. They all returned to the tunnel a minimum of 10 times in 1 hour with a minimum of 3 minutes between 2 passes.

As we focused on the integration of optical invariants, which are task specific, we had to consider only bees performing the given task; the ground-following task. Performing a ground-following task involves flying at a constant altitude by collecting optical information from the ground. We considered bees were likely to perform a ground-following task as soon as they spent less than 500 ms below altitude of 30 cm. In each experimental condition, 13 trajectories were randomly selected among the ones respecting this criterion.

B. Optical manipulations

Once bees were familiarized with a specific visual configuration, the recording phase of the study could begin. The methodology used consisted of recording the flights of bees in a number of successive trials reproducing the visual configuration used in the familiarization phase (catch trials), interspersed with a limited number of trials in which a specific invariant was manipulated.

The current study pursues an essentially methodological ambition and we aimed to ensure that our experimental set-up allowed the recording of altitude adjustments in bees following the manipulation of a specific optical invariant. The contribution of the two invariants in altitude control will be explored in a future study through a series of experiments in which the behavior of the bees will be examined in more or less impoverished environments when the two invariants provide contradictory information.

Going back to this study, we wanted to examine the behavior of bees confronted by SARC manipulations that occur when hovering over poorly-textured ground. More precisely, the motorized rods were set in motion manually 2 seconds after the bees entered the tunnel. Each bee was about halfway through the tunnel when this perturbation occurred. Their trajectories were recorded since they entered the tunnel.

In the converging condition, the rods originally positioned at the floor-wall junction (Fig. 2-B.i) were set in motion such as to give rise to an increase in splay angle (Fig.2-C). The diverging condition exposed the bees to the opposite stimulation, with a decrease in splay angle (Fig.2-C). Our experiment included two control conditions in which the bees' flight was recorded in the absence of SARC manipulations. For the converging condition, the rods remained immobile in parallel position (Fig. 2-B.i), the whole crossing. In the diverging condition, the rods remained immobile in a narrow position (Fig. 2-B.ii). In our experiments, one end of the rods took 200 ms to cover 10 cm, half of the screw length, corresponding to an angular velocity of 14°/s with an angular displacement of 2.8°. During the whole experiment, bees circulated freely within and outside the tunnel. They took minimum 3 min to return to the tunnel entrance (see section 5.A), so a SARC manipulation was never applied for more than 3 minutes to avoid a bee encountering the perturbation twice in a row.

C. The decorrelation issue between the two optical invariants: SARC & OSRC

In our experiment the moving rods have been used to manipulate the SARC. In accordance with our objective, the average rate of change of the splay angle induced by the movement of the rods was 14°/s and -14°/s in the converging and in the diverging conditions, respectively. Nevertheless, strictly speaking, moving rods do not only specifically manipulate the SARC but can also affect the OSRC. The movement of the bees above the ground in the tunnel gives rise to a translational OF (Fig. 3-A). As an example, the OS of the point on the ground closest to the bee is 3.5 rad/s for a bee flying 17 cm above the ground at a velocity of 0.60 m/s. Moving rods will add to this purely translational global OF resulting from the flight of the bees, 'local' components resulting from rods' motion (whether convergence Fig. 3-B or divergence movements Fig. 3-C). A close inspection of figures 3-B,C allows a better understanding of the presumed impact of rods' movements on the resulting OF. First of all, local motions do not change the overall OF pattern. In the case of bees relying on a weighting process of all optical flow velocity vectors to navigate safely in the environment (as suggests [8] in bumblebees), local motion would have minimal impact on the produced trajectories. Secondly, the local OF components close to the bee are of an amplitude equivalent to the vectors corresponding to the global OF, providing consistent information. Finally, the local OF elicited by moving rods is of the same amplitude whether it is a convergent or divergent motion so that there is no reason to expect different regulations in the two conditions (i.e., converging and diverging conditions). Taken as a whole, these different elements point to a presumed marginal impact of the moving rods on the perceived OS.

D. Predictions

In the case SARC is used to control bees' altitude, the converging condition should give rise, once the manipulation is initiated, to an increase in altitude (Fig. 2-C), the diverging condition should give rise to a decrease in altitude, while the altitude should remain unchanged in the control condition (Fig. 2-C). An increase in altitude is anticipated in the converging condition because, when the laws of physics are not violated, an increase in splay angle specifies a loss of altitude which must be compensated by an appropriate regulation. The reverse is true in the diverging condition, i.e., the optically specified increase in altitude should be compensated by a decrease in altitude, while there is no reason to anticipate an altitude change in the control condition.

E. Statistical analysis

Linear mixed models (lmer) using the lme4 package [27] were developed to test the influence of SARC manipulations on flight altitude. Individual identity was included as a random factor, to account for individual variation. When main effects were found significant, emmeans comparisons (Emmeans package) were used to compare altitude binning distribution by pairs: last bin before perturbation (4th) versus each bin after perturbation inside the same condition (parallel condition before *vs.* after perturbation, converging condition before *vs.* after perturbation, etc...). Pairwise comparisons between every bin inside each of the two control conditions (parallel or narrow) confirm that bees' altitude was usually straight in the absence of perturbation. All statistical tests were performed using R software [28].

6. RESULTS AND DISCUSSION

A. Results

In this experiment we focus on the ground-following behavior produced by bees flying over poorly-textured ground while the SARC can either be manipulated or not. The analyses focus on the time course of altitude changes in the three experimental conditions (Fig. 4 and some raw trajectories from the trials are visible in Fig. S2). In the control condition no manipulation occurs. The results reveal that the bees pass through the tunnel without producing a change in altitude (Fig. 4-A ; for all comparisons $p>.01$). In the converging condition the rods are set in motion 2 seconds after the bees enter the tunnel. The results reveal an increase in altitude 450 ms after the onset of the manipulation (Fig. 4-A ; $p<.01$ when comparing the altitude just before the manipulation and 450 ms after the manipulation). In the diverging condition the

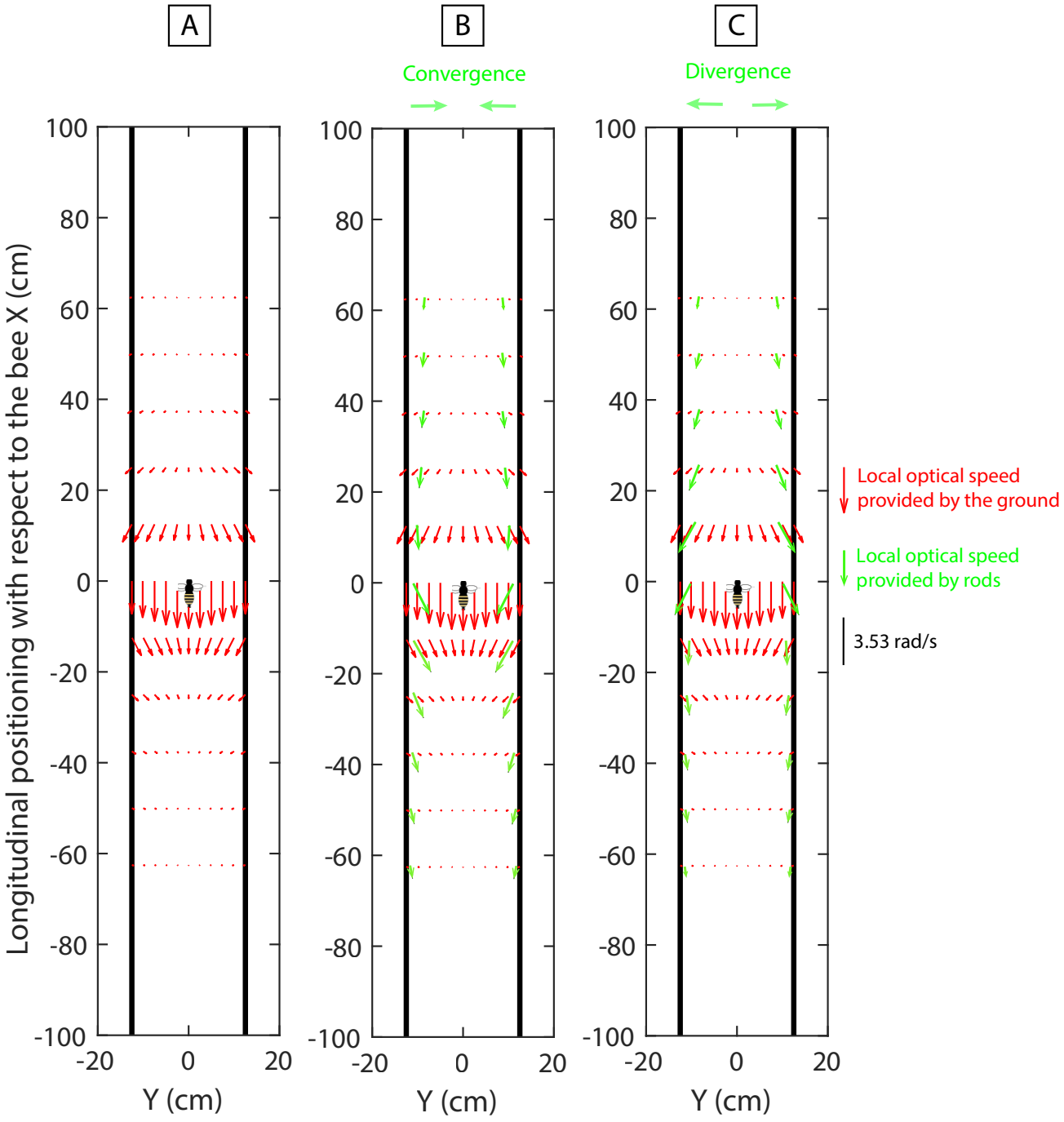

Fig. 3. Local optical speed provided by rods compared to global optical speed provided by the ground to a bee flying halfway through the tunnel. Arrows represent true to scale local optical speed provided to a bee flying 17 cm over the ground at a velocity of 0.60 m/s. The resulting optical speed coming from the ventral part is generated by the combination of the optical speed coming from the ground (red arrows in **A**), and the local optical speed generated by converging (green arrows in **B**) or diverging (green arrows in **C**) rods.

rods are again set in motion 2 seconds after the bees enter the tunnel but this time in a divergent way. The results reveal that the bees pass through the tunnel without producing a change in altitude (Fig. 4-B ; for all comparisons p>.01).

B. Discussion

The ambition of this work was to validate an experimental setup designed to determine to what extent bees can use different optical invariants simultaneously in order to control their altitude. In this perspective, an experiment was

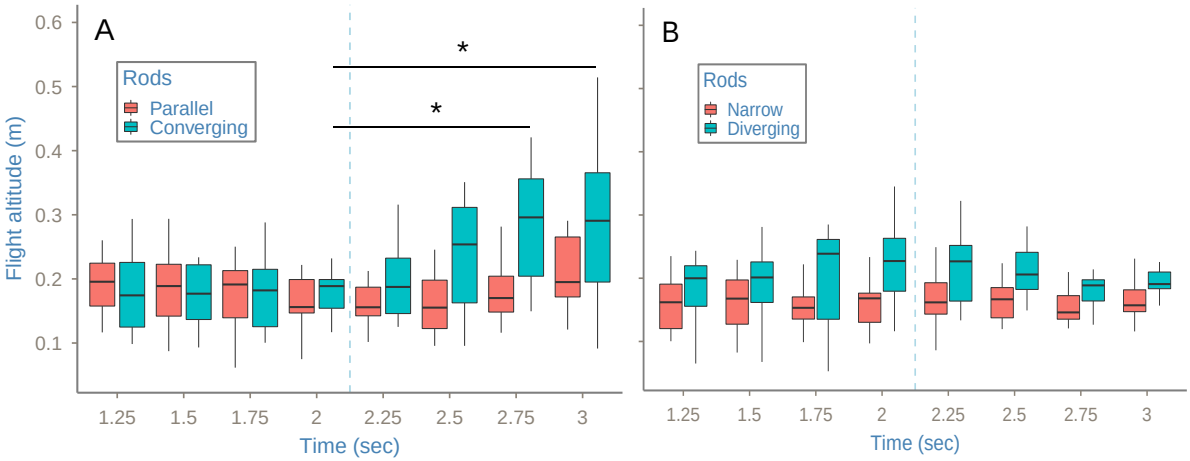

Fig. 4. Time course of altitude changes in the two experimental conditions (**A**-converging condition ; **B**-diverging condition) and their respective control condition (parallel and narrow). While the altitude remains unchanged in both the control conditions and the diverging condition, an increase in altitude can be observed in the converging condition starting from 2.75 s after the perturbation (blue dotted line). 13 randomly selected flights were analyzed in each condition, respecting a ground-following criterion (see section 5.A for selection criterion). * means altitude difference is statistically significant ($p<.01$). NS means the difference is not significant ($p>.01$). Some raw trajectories from the trials are visible in Fig. S2.

carried out allowing the SARC to be biased unexpectedly, thanks to motorized rods, during the flight of the bee over a poor-textured surface.

The results revealed an increase in altitude when the splay angle increases (converging rods conditions) while the bees' altitude was maintained unchanged when both the splay angle was not manipulated (control conditions) and when the splay angle decreased (diverging condition). The increase in altitude is compatible with the use of the SARC by bees to control their altitude. Logically, the decrease of the splay angle (diverging condition) should have elicited a drop in altitude, which is not the case. Now, in the current experiment, bees entered the tunnel 14 cm over the ground and even if the ground was poor-textured it still provided information related to OS; otherwise the bees would collide with the ground [9]. We could hypothesize that in the case of a conflict between invariants (in our experiment the SARC tells the bee to reduce altitude while OSRC tells the bee to maintain altitude) a security principle could operate giving priority to the invariant endanger the bee as little as possible, i.e., avoiding being too close to the ground.

Obviously, additional experiments will be necessary in order to better understand the rules and principles that prevail in the integration of invariants in bees. Our experimental setup is a tool of choice for carrying out these studies. Optical invariants can be withdrawn or biased in the presence of more or less impoverished environments, while task constraints (e.g., flight height) can be easily manipulated. This first study revealed however the potential of our setup because we were able to show that another invariants that the OSRC could be taken into account by bees to control their altitude.

VR platforms potentially offer a powerful tool to identify the perceptual mechanisms underlying flight control in bees as it allows easy control and manipulation of the visual scene. Nevertheless, these platforms also suffer from a number of shortcomings in the forefront of which is the fact that insects' behaviours are analysed far from the environment in which they are carried out. Under current technological limitations, the VR platforms used for experiments on insects are not totally immersive (e.g., the dorsal information is missing [29], also, impoverished artificial light is used, making the transposition of the results obtained, to real life, hazardous (except for the most advanced research in ants [30] or in bees [31]). Obviously, our experimental tunnel also has its share of limitations, such as a restricted range of manipulation of SARC due to its width, which would be very easy to handle in VR. Far from being able to replace VR platforms, there is clearly a need for the kind of setup presented in this manuscript. Allowing for simultaneous precise experimental control and ecological validity, it could firmly corroborate or nuance results obtained in less ecologic environments.

7. CONCLUSION

In conclusion, our setup can be said to be particularly innovative from different points of view: not only has it been designed to answer theoretically grounded questions in 'ecological entomology' [10], but it also allows a precise control of the informational content of the environment while preserving the visual environment of bees or any other flying insect. Our middle term ambition would be to identify and to model optical invariants-based universal control principles that could provide robust control algorithms for flying robots [6]. We hope our contribution could, to some extent, pave

the way for a future generation of 'ecologically inspired' studies that would participate in the debate of ideas among researchers in the entomologist community.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

A.B.D.: investigation, methodology, resources, software, visualization, writing—original draft and writing—review and editing. G.M. and J.R.S.: conceptualization, funding acquisition, investigation, methodology, project administration, supervision, validation, writing—review and editing. All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

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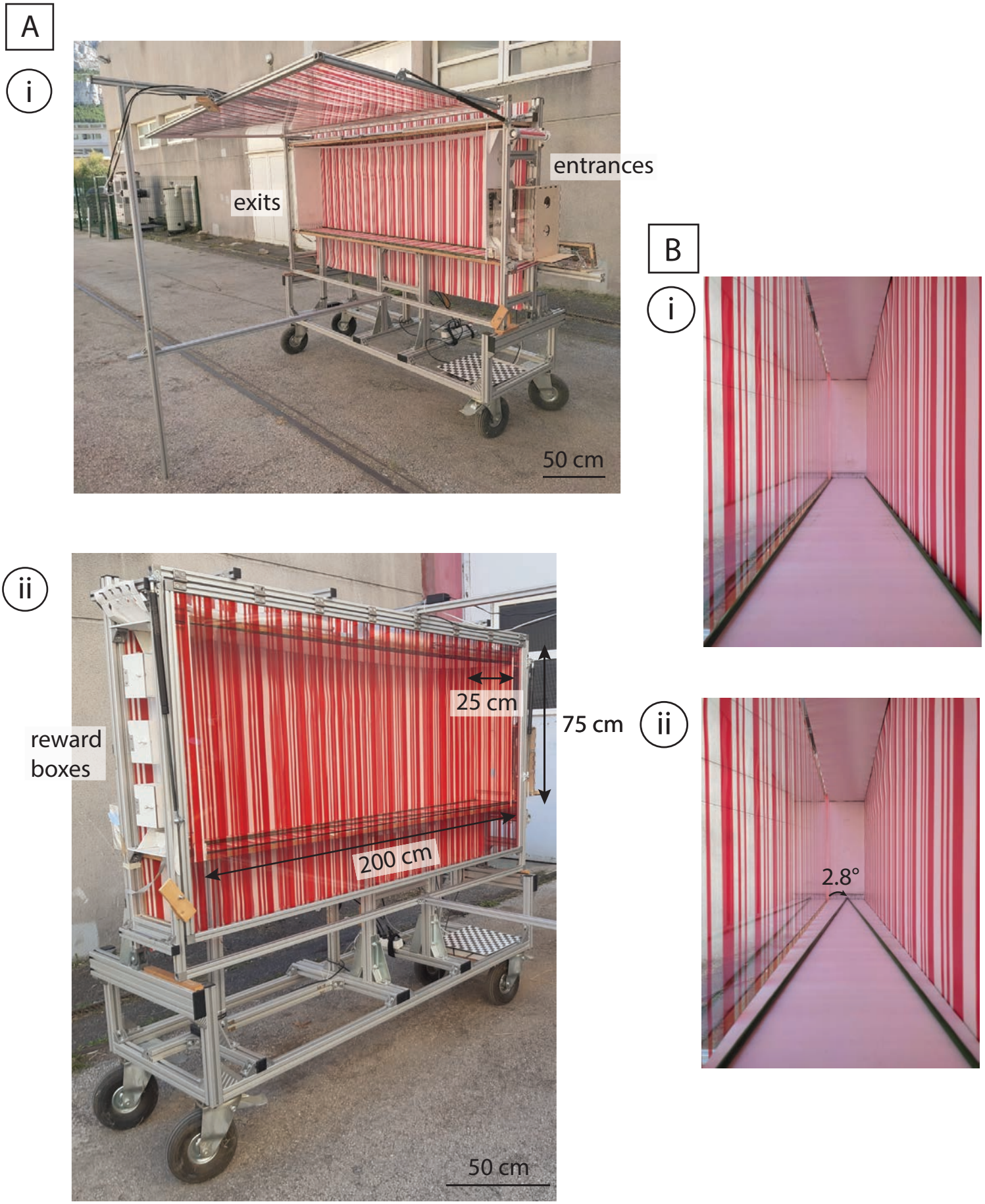

Fig. S1 - Photographs of the apparatus: (A.i) and (A.ii) are views of the whole tunnel, (B.i) and (B.ii) are views from inside the tunnel. (A.i) shows the inside of the tunnel with the open plexiglas wall. (A.ii) Shows the tunnel with the plexiglas wall closed, as placed during the experiments. On both views at the tunnel entrance was installed the RFID tracking system, which was not used for the experiments. (B.i) shows the parallel rods and (B.ii) shows the narrow rods at the entrance altitude of the bees. The set-up was located at coordinates 43°14'02.2"N 5°26'38.3"E. Hives were located at 60m from the apparatus Experiments were conducted between 31/08/2021 and 27/09/2021.

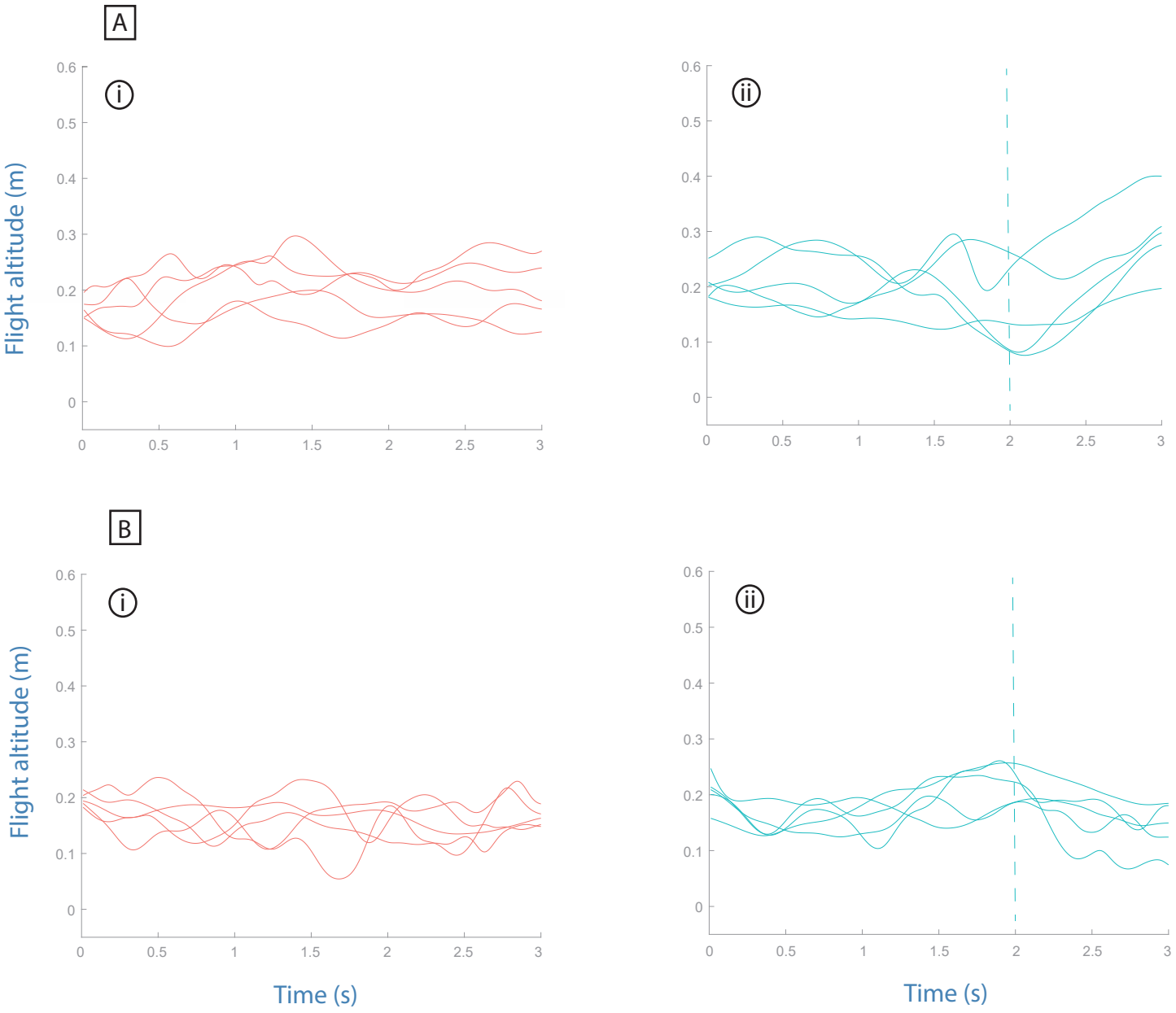

Fig. S2 - Individual time course of altitude changes in the two experimental conditions: A.i and B.i are control conditions, A.ii is the converging condition and B.ii is the diverging condition. While the altitude remains unchanged in both the control and diverging conditions, an increase in altitude can be observed in converging condition after the perturbation (blue dotted line). 5 randomly selected flights are plotted in each condition, each one respecting a ground-following criteria (see section 5.A). The data was acquired at 100 Hz, then low-pass filtered by a second-order Butterworth filter with a cut-off frequency of 3 Hz to smooth trajectories.