**Editorial comments:**

•All of your previous revisions have been incorporated into the most recent version of the manuscript. In addition, Editor may have made minor copy edits to your manuscript and formatting changes to comply with the JoVE format. Please maintain these changes. On the JoVE submission site, you can find the updated manuscript under "file inventory" and download the microsoft word document.  **Please use this updated version for any future revisions and track all changes using the track changes function in Microsoft Word**.  
  
•Formatting:  
-Line 191: “Confirm the plume width by using TiCl4 smoke prior to the experiment” – this action occurs out of time sequence, and therefore would be best conveyed as a note. I.e. “Note: Plume width should be confirmed prior to the experiment by using TiCl4.”

Response: We have modified as the editor’s suggestion and put this action out of time sequence (step section 5.2).

-Please include spaces between all paragraphs.

Response: We have included spaces between all paragraphs.

•Grammar:  
-2.3 - “The adhesive does not touch…” should be “The adhesive should not touch…”  
-2.6 – ““Hold the tethered moth at a stand and put a piece of paper under the legs to give it a rest before getting inside the cockpit of the robot.” Rewrite to “…under the legs to rest the moth, before placing it inside the cockpit of the robot.”

Response: We have revised those sentences (step sections 2.4 and 2.6).

-Line 179 – “Any contaminations”

Response: We have revised “Any contamination” (step section 4).

•Additional detail is required:  
-1.1-1.3 is there a way to sex silk moth pupae? Can you provide representative images? Or are all pupae just kept together until eclosion, and then separated?

Response: We discriminate males from females at pupal stage, based on the sex marks on the abdominal tip. We have added the explanations and representative images in step section 1.2 and Figure 1.

-3.2 clean the surface with what? Do you use anything in particular to clean the ball?

Response: We wash the ball with water. We have added the explanation (step section 3.3).

-3.3 is there any particular speed, power or setting for the blower fan to maintain hovering at 2mm?

Response: We have added “the fan is supplied by 9 V” in step section 3.4.

-3.5 is there an example height that seems to work well? Assuming an average sized male moth?

Response: It is difficult to show an example of appropriate height, but the important points are (1) keep the same height of the ball before and after attaching the moth, and (2) observe its walking behavior in response to the pheromone. We have described these points in step 3.6, and have added a protocol for the preparation of a pheromone stimulus cartridge in step 4.1, which is used for triggering walking behavior of the moth.

-6.1.1 – How is the file edited?

Response: The file which defines the motor gains is a text file, therefore it can be edited by a text editor. We have added this explanation in 6.1.2.

•Please take this opportunity to thoroughly proofread your manuscript to ensure that there are no spelling or grammatical errors. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.  
   
•Please disregard the comment below if all of your figures are original.  
If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."   
  
**Reviewers' comments:**

**Reviewer #1:**  
*Manuscript Summary:*  
In 'Insect-controlled robot: a method to evaluate odor-tracking performance of a future insect-mimetic robot' the authors describe a mobile odor-tracking apparatus that is controlled by an on-board silkmoth. The advantages of having an insect control the apparatus are that: 1) The behavior of the 'robot' when under the control of the silkmoth can be compared to behavior of a robot under self-control in order to identify successful odor-tracking strategies. 2) Sensory inputs and motor outputs of the moth can be modified to investigate what input/output mappings are being used by the moth for odor tracking.  
  
*Major Concerns:*  
-The paper is well written and clear, and certainly describes an interesting idea for investigating both biological and artificial odor tracking. However I'm unsure if it is suitable for publication in JOVE. The most fundamental and vital apparatus for all of the experiments described in the paper is, of course, the robot itself and the computer program used to interface with and control it. I understand that the technical details describing how to build the robot would be long and complex, but without these details it is absolutely impossible for anyone to reproduce these experiments. From my understanding, the ability to reproduce the experiments in the primary focus of JOVE.

Response: As the reviewer commented, the ability to reproduce the experiment is the primary focus of JoVE. However, it is difficult to describe throughout experimental system in particular if the system is a custom-built. In this article, therefore, we described a basic design and characteristics of the robot in the text and will focusing on showing how it works in the JoVE video format. The spherical treadmill is a well-known method for analyzing animal locomotion and related papers have also published in JoVE, therefore, the robotic part should be well described in the text. We have added explanations about the transformation from insect locomotion to motor rotations in step section 3. We have also added several important components of the robot in the table of materials and equipments

-Section 5: Odor source localization experiment  
No information is provided on construction of the robot. The section contains minor details like names of interface buttons but is missing any information on how to build the robot or write the computer program to control it.

Response: We have added explanations in detail, focusing on the basic design of the robot and how to control its parameters from a computer program in step section 3.

-Step 5.7) The function of the button "param1" is not defined anywhere and is meaningless without the computer program that controls the robot.  
-Step 6.1.2) The name of the button "param2" is meaningless without the computer program that controls the robot.

Response: We have added explanations describing what these buttons mean in step sections 5 and 6.

*Minor Concerns:*  
-Step 6.4.2) This step actually inverts the motor output of the robot, not the visual input of the moth. There is a large difference between these two manipulations and they shouldn't be confused.

Response: We have corrected “to invert the motor output” (step section 6.2).

-Figure 6: The placement of the odor source should be illustrated in the figure.

Response: We have illustrated the location of the odor source in Figure 8 (figure 6 in original manuscript).

-L388: If roughening the floating polystyrene ball is important (as stated in Discussion) then it should be mentioned in the methods.

Response: We have added a note in step 3.3.

-L392: If the moth habituates to the odor then this should be mentioned in the methods.

Response: we have moved this sentence to step section 3.6.

-L121: 'which' should be deleted  
-L123: '40 m' should be '40 mm'  
-L333: 'allows' should be 'arrows'  
-L342: do the authors mean 'left side' rather than 'lateral side' (which literally means 'side side')  
-L413: 'hight' should be height

Response: We have corrected them.

*Additional Comments to Authors:*  
N/A  
  
  
**Reviewer #2:**  
*Manuscript Summary:*  
This manuscript introduces an interesting insect-controlled robot for odor tracking using silkmoth's inborn ability to move towards pheromone. The content is well organized and the experiment protocol is elaborately illustrated. The result shows that this insect-driven robot car is capable of tracking designated odor and the tracking performance is related to the direction as well as distance of the two tubes. However, there are some shortcomings that need to be further illustrated.  
  
*Major Concerns:*  
1. The manuscript seems to be similar to a previous work of the authors (Ando N, Emoto S, Kanzaki R. Odour-tracking capability of a silkmoth driving a mobile robot with turning bias and time delay[J]. Bioinspiration & biomimetics, 2013, 8(1): 016008.). I think it might be necessary for the authors to show clearly the differences between these two researches and the novelty of the present one.

Response: The JoVE publishes expanded descriptions of techniques that have previously appeared in results-based journal. Therefore the manuscript is based on our previous papers. The main focus of this article is to provide detailed information about our experiment using the insect-controlled robot, which should be the difference between this manuscript and previous ones. We have added detailed description about the robot in step section 3.

2. The application of this method seems quite limited now. The authors are expected to illustrate how does this method work if the odor sources are not interested by the moth but are significant for human beings, such as explosives and drugs?

Response: It is a very important point. Our colleagues succeeded to generate transgenic silkmoth which express different olfactory receptors on the pheromone receptor neurons. If we avail such transgenic silkmoths that respond to some characteristic chemicals of explosive or drugs, and use them as pilots of the insect-controlled robot, our method would be a useful robotic platform to find these chemicals. We have described this point in the last paragraph in discussion.

*Minor Concerns:*  
3. The manuscript and pictures show that there are two separate air circuits in the robot (one to support the ball in cockpit, the other to collect odors from the outside environment). Is it necessary to isolate these two air flow, and if so, how to isolate them in the robot?

Response: The air intake for the air supplying the spherical treadmill must be separated from those for the odor delivery. Without the separation of air intakes, the pheromone contaminates into the air supplying the treadmill, which may apply non-directional odor cues to the moth. This point was omitted in the original manuscript so that we have added the explanations in step section 3.1.

.   
4. In Fig. 7A, the authors show the result of tracking the moving trajectories of silkmoth, but no such technique is introduced in the manuscript.

Response: The data is from our previous study, which is cited in the figure legend. The method was a conventional method, simply tracking the center of gravity of a moth in each frame after the experiments. We therefore think that it is not necessary to describe in the manuscript.

5. In Fig. 7 and 8, the goal area is indicated by a circle. How big are the circles to determine that the robot has found the odor sources?

Response: The radius of the goal area was defined as the length between the moth and the tip of odor suction tubes. Because each trajectory indicates positions of an onboard moth, the radius is approximately equivalent to the closest distance between the onboard moth and the odor source. We added this explanation and citation in the legend of Figure 10.

*Additional Comments to Authors:*  
6. In the test session, wind is exerted to the experiment tunnel. What if no wind is applied? Can the robot find the odor source simply by volatilization? The research would be better if some interference odors are involved to testify its odor tracking ability.

Response: We thank the reviewer for the valuable comment. We have been conducted the odor tracking experiments only in a windtunnel where the odor flow is well-controlled. As the next step, we will test in odor circumstances without wind or with turbulence, which would be an important step to investigate the insect odor tracking capability in a real environment.   
  
**Reviewer #3:**  
*Manuscript Summary:*  
The authors present a method for controlling a wheeled robot with a silkmoth as a pilot walking on a rubber ball that controls the movement of the robot. The rotations of the ball are tracked using an optical sensor and this information is translated into wheel movements. They explain the steps through the experiments for building the robot and evaluating its behavior. They then compare odor tracking performance of this robot to a walking silkmoth performing the same task. This allows evaluating the behavior of the robot in response to odor detection by the moth.  
  
*Major Concerns:*  
The experiments are very interesting but the authors are narrowing down the possible impact of the paper by presenting it as an evaluation method for a future bio-mimetic robot the way they emphasized in the Title and the Abstract. On the other hand in the Discussion they mention the tasks fulfilled for evaluating odor tracking capability of insects, but not a bio-mimetic robot. Therefore it is not clear to me whether the main rationale is to evaluate a bio-mimetic robot or an insect. Moreover, the method presented here cannot be a direct and sole evaluation of a future bio-mimetic robot as claimed in the Title and the Abstract, as the authors are only evaluating the the motor behavior of a robot performing an odor tracking task, without considering the problems that may arise related to any possible sensory acquisition performance. This does not hinder the quality of the paper and I understand that this method is a step forward in evaluating a bio-mimetic robot, but it would be more useful either if they could present the paper in this direction mentioning in what sense this method provides an evaluation of a bio-mimetic robot, also briefly discussing what other questions should be addressed for evaluating a bio-mimetic robot (like use of chemosensors, control program etc.) not only in the Introduction but also in the Discussion, or change the Title to a more explicative one rather than discussing the future goal or a possible application field of the paper in the Title.

Response: We thank for the reviewer’s suggestion. As the reviewer pointed out, the title and the former part of the original manuscript focused on biomimetics perspective, which was not consistent throughout the manuscript. We have changed the title as “Insect-Controlled Robot: A Mobile Robot Platform to Evaluate Odor-Tracking Capability of an Insect” for covering broader perspectives and have modified long abstract and introduction.

- It is not clear to me what the authors mean by "manipulation of time delay" in 6.2 (line 237). This is the delay between what, and how is it controlled? Is it about sending the signal to the robot after a movement is observed by the optical sensor? Neither the motivation for applying a time delay, nor its implications on behavior are discussed in the paper.

Response: The time delay means the response delay of the robot movement. The aim of this manipulation is to investigate the acceptable period of time spent for sensory-motor processing which would be helpful information for the biomimetic odor tracking. This manipulation was achieved by storing locomotion data into a buffer memory during the specified time delay. We have added the explanations in step section 6.3.

- It would be clearer if the positions in Fig. 5 are mentioned in quotes in the text (such as '"good" position' instead of 'good position'), or to enumerate the three positions as A,B,C and refer to the positions with these labels.

Response: We have enumerated three positions in alphabetic order in Figure 7 (originally Figure 5).

- In Fig. 7, it is not clear to which trajectory the yellow trajectories correspond to. There does not seem to be any yellow lines that correspond to the red trajectory in B for example, unless the wheel rotations (if any) of the robot allow it to move in a direction where it is not facing the odor plume. Are all the 7 trials shown with yellow traces?

Response: All yellow lines including those belonging to the red line are overlaid. Because the silkmoth often perform pinwheeling, it might be difficult to find the corresponding trajectories of the yellow lines (tube tips). We presented the yellow lines to show the difference in searching areas between a small-sized silkmoth and the large robot. However, there are so many lines and it is hard to discriminate each of them. Therefore we have deleted the yellow lines in the revised manuscript.

- How is the gap between the tubes controlled? Is the distance measured between the tips? Does the gap length have an effect on the angle between the tube entrances and the odor plume direction?

Response: The gap indicates the distance between the odor suction tubes on the left and right. Either the wide (90 mm) or narrow (20 mm) gaps can be set. It is sure that the change of the tube gap alters the angle of the tube entrance, but their effects on odor acquisition have not yet been evaluated. We checked the air flow around the tube tip with TiCl4 smoke, which showed that the different tube gaps effectively alter the odor sampling areas (Ando, N. & Kanzaki, R. J. Exp. Biol. 218, 3845-3854, doi:10.1242/jeb.124834 (2015)).

*Additional Comments to Authors:*  
There are some ambiguous sentences, such as "The concept of this robot is simple: the evaluation of the performance of insect-  
mimetic odor tracking is possible if an actual insect, instead of bio-mimetic models,  
drives a robot." in the Abstract. The authors should be more explicative when making such statements.

Response: This sentence has been deleted in the revision of long abstract according to the major comment.  
  
**Reviewer #4:**  
*Manuscript Summary:*  
Ando et al. presents a very cool apparatus to manipulate environmental cues involved in odor tracking by insects. The authors find that a moth driven robot can track an odor plume and less so when bilateral odor cues are altered or if motor output is inverted. Overall, the study is clear and provides an interesting bio-robotic interface to ask questions about odor localization. Furthermore, the discussion describes pitfalls and limitations and puts the apparatus in the broader context of research on olfactory search behavior. I think the story is solid and should be published with minor revision.  
  
*Major Concerns:*  
N/A  
  
*Minor Concerns:*  
-I think the manuscript could add appeal and breadth by considering and lightly discussing Braitenberg vehicles and olfactory search (See Gomez-Marin et al.). Obviously the apparatus has important parallels and considerations in light of such speculation could be fruitful. Also, there is little discussion of the results in 8D. Are there any studies that report a deficit in insect odor tracking when visual input is manipulated or removed?  
-Gomez-Marin A, Duistermars B, Frye M.A, Louis M. Mechanisms of odor-tracking: Multiple sensors for enhanced perception and behavior. Frontiers in Cellular Neuroscience. 2010;4(6)

Response: We thank the reviewer for the valuable comment, and the insect-controlled robot can be used for this aim. We have added the explanation and the citation in the last paragraph of discussion. Regarding the significance of visual input for odor tracking, we reported in the following papers. The former reported the optic flow input is required for compensation of turning bias, and the latter reported that the self-induced optic flow acts as reafference and is compared to putative collorary discharge signals that encodes surge directions. We did not discussed in detail about Figure 11, but have cited the reference at the end of the last paragraph in representative results.

1) Ando, N., Emoto, S. & Kanzaki, R. Odour-tracking capability of a silkmoth driving a mobile robot with turning bias and time delay. Bioinspir Biomim 8, 016008, doi:10.1088/1748-3182/8/1/016008 (2013).

2) Ando, N. & Kanzaki, R. A simple behaviour provides accuracy and flexibility in odour plume tracking – the robotic control of sensory-motor coupling in silkmoths. J. Exp. Biol. 218, 3845-3854, doi:10.1242/jeb.124834 (2015).

-Line 84: "Because of the lack of the models, it has been difficult to estimate regarding the performance of such future insect-mimetic robots." - confusing, please revise.

Response: We have revised this sentence to make clear the problem presentation as “Therefore it is still unknown how such a biological system actually works as a controller of a robotic platform.”

-Line 97: change "…of the future inset-mimetic…" to "…of the future insect-mimetic…"

Response: This word has been deleted in the revision.

-Line 140: change "The adhesive does not…" to "The adhesive should not…"

Response: We have changed.

-Line 144: what does "Hold the tethered moth at a stand…" mean?

Response: This is a step to keep the moth tethered before putting it inside the cockpit. We have revised as “Keep the moth tethered before placing it inside the cockpit of the robot. Hold the attachment at a stand and …” in step section 2.6.

-Line 155: change "Design the hardware…" to "Design of the hardware…"

Response: We have not changed because "Design the hardware…" is based on the format of protocol section in JoVE.

-Line 168: change "…the middle legs is at…" to "…the middle legs are at…"  
-Line 257: change Figure 7D to Figure 8D  
-Line 263: change "significances" to "significance"  
-Line 293: change "…robot continued to circling…" to "…robot continued circling…"  
-Line 333: change "Red allows…" to "Red arrows…"  
-Line 456: Cite: Duistermars BJ, Chow DM, Frye MA. Flies require bilateral sensory input to track odor gradients in flight. Curr. Biol. 2009 Aug 11; 19 (15):1301-7

Response: We have corrected these errors and added the citation.

-Figure 8: please indicate gap width, inversion of tubes and motor output with cartoon vehicles in B-D (as in A).

Response: We have added cartoon vehicles in Figure 11B-D (originally Figure 8).

*Additional Comments to Authors:*  
N/A