Bumblebees (*Bombus terrestris*) Raise Their Legs Regularly in Response to Specific Stimuli

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Abstract

Bombus (one of the species called bumblebees), which range throughout Japan, play important roles for plants as pollinators. In some kinds of *Bombus*, the reaction in which they raise their middle or back legs is known, however, the detail of the reaction has not yet been solved. The authors set the following aims; determine the adequate stimulus and frequency of the reaction, and estimate the ultimate factor of the reaction. We used vibrational and visual stimuli on the individuals of *Bombus terrestris* (materials of our study), and observed them in the laboratory. As a result, we found that they raise their legs regularly in response to each of the stimuli we mentioned above. Furthermore, during our observation, we also determined the frequency for each stimulus and patterns of the reaction. The conclusions from the result of the experiments and discussion are that the vibrational and visual stimuli are adequate to produce the reaction, the frequency of the reaction varies between each stimulus, and more inspections are needed to estimate the ultimate factor of the reaction.

Introduction

Bombus (one of the species called bumblebees) play important roles for plants during spring to summer as pollinators, and they are familiar to humans. It is considered that 250 species of *Bombus* range around the world, and in Hokkaido, 12 species including *Bombus terrestris*, which is designated as invasive alien species by the Ministry of the Environment from 2006 range.

In June 2020, the authors picked a queen bee of *Bombus diversus tersatus*, and observed indoors. At the time, we noticed that it raises its legs unnaturally when we

Fig.1 the individual of *Bombus* in the field

approach its rearing box or move it. Therefore, we picked other species of *Bombus*, and observed these. As a result, we found that they react same as a queen bee of *Bombus diversus tersatus.*

Fig.2 the reaction of *Bombus terrestris*

We looked for preceding studies on this reaction, however, we could not find any papers. According to one website, Hans-Jürgen (2002), states that the individuals of *Bombus* raise their legs when humans approach them as defense or warning. Furthermore, Brodie (2020), states that they raise their one middle leg when they feel threatened. However, these are the hypotheses of the factors by observation in the field, and these two authors did not investigate the reaction. Moreover, they did not mention the conditions which lead to the reaction.

The aims of this study are; determine the adequate stimulus and frequency of the reaction, and estimate the ultimate factor of the reaction.

As we mentioned above, we can observe the reaction when we approach the rearing box or move it (specifically, when we opened the lid of the rearing box or brought our hands close to the individuals of *Bombus*). From these observations, we set two hypotheses; the vibrational stimulus when objects collide with the container with the individuals of *Bombus* is adequate for the reaction (referred as hypothesis 1 in this paper), and the visual stimulus when objects approach the individuals of *Bombus* is adequate for the reaction (referred as hypothesis 2 in this paper).

In general, the species of *Bombus* are called bumblebees comprehensively, however, the following of this paper, we call the individuals of *Bombus terrestirs* as bumblebees for convenience.

Materials and Methods

Materials

In July 2020, the authors picked a nest of bumblebees, and we used inside individuals as materials of this study. As we mentioned above, this species is designated as invasive alien species by the Ministry of

Fig.3 the nest of the bumblebees we picked

the Environment, therefore, in principle, it is prohibited to breed or carry them. In consequence, we got permission to use them as materials of this study from the Ministry of the Environment (License No.20000141).

Rearing box

The authors used a plastic box 16cm in length, 35cm in width, and 23 cm in height as a rearing box. We removed lagging material, and put bumblebees (50 worker bees and a queen bee) into the rearing box to breed in the laboratory.

Fig.4 the rearing box with license from the Ministry of the Environment

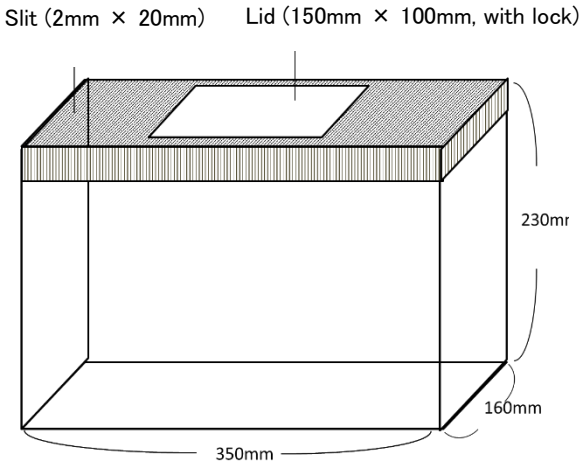

Fig.5 schematic diagram of the rearing box

Breeding environment

The temperature of the laboratory was 20 to 30 degrees Celsius, and the illuminance of the laboratory was about 500lux. The authors kept dry pollen 10g+2g or 10g-2g in the rearing box all the time, so that bumblebees can take it. Furthermore, we fed 4g of honey every day.

Fig.6 the dry pollen and honey

Preparation for the experiments

Definition of the reaction and recording technique

The authors considered the definition of the reaction, and it was as follows; if bumblebees take action which they raise their legs higher than parallel lines to the ground from their joint of legs during they are taking their normal balance, we defined it “the reaction”, and called as “bumblebees reacted.” In this case, we recorded which legs (left or right, and front or middle or back) caused the reaction. Except the actions we mentioned above, we defined it “the non-reaction”, and called as “bumblebees did not react.”

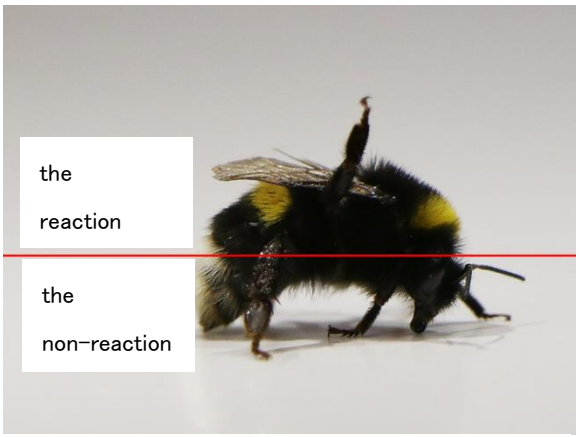

Fig.7 the definition of the reaction

Environment of the laboratory

As the authors mentioned above, we adjusted to keep the temperature of the laboratory 20 to 30 degrees Celsius, and the illuminance of the laboratory about 500lux.

Experiment container

The authors spread a white cloth in a plastic cup 5cm in diameter, and 3.5cm in height. We put each bumblebee (a worker bee) into this, and used as an experiment container. Furthermore, we adjusted that bumblebees are 5mm away from the wall of it, stop their movement, and take their normal balance.

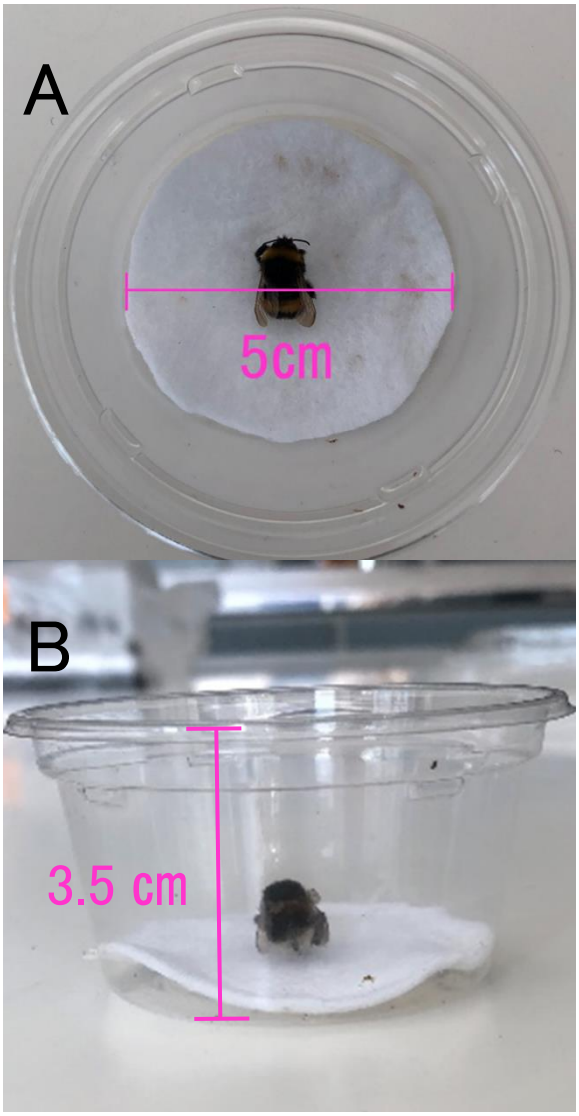

Fig.8 the experiment container from the (A) top and (B) side

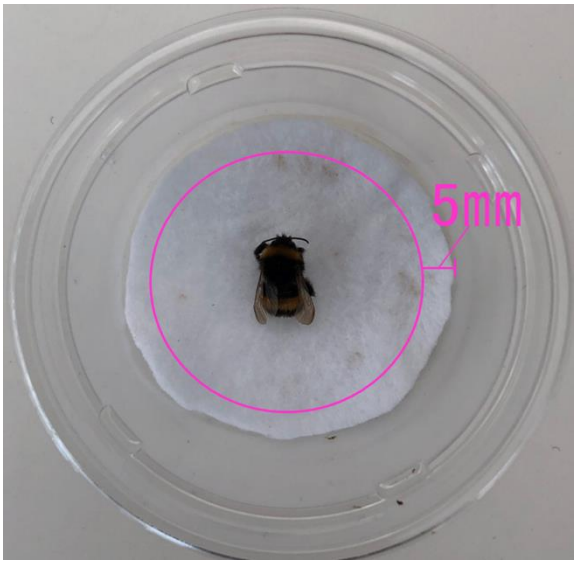

Fig.9 the experiment container after we adjusted

Times of the experiments and interval of succession times

The authors conducted each experiment three times. The interval of succession times (between first time and second time or between second time and third time) was as follows; if bumblebees reacted, we waited until they get their legs down, and conducted the experiment of the next time 10 seconds later. On the other hand, if bumblebees did not react, we conducted the experiment of the next time 10 seconds later.

Experiment 1 (testing of hypothesis 1)

The authors set the experiment container close to the arm of the iron stand 50cm in height.

We dropped a globe of styrene 2.3cm in diameter, and 0.3g in weight tied with thread freely to the lid of the experiment container. At the time, we adjusted the position of the experiment container that the globe collide with the lid of it.

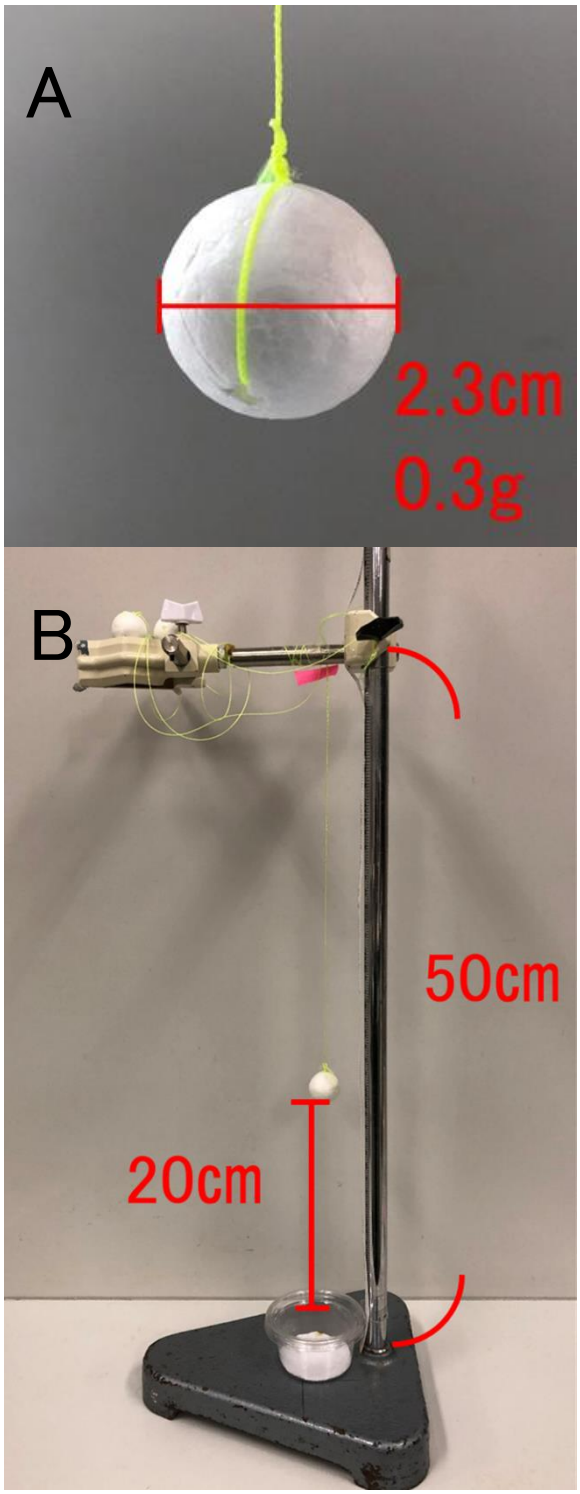

Fig.10 (A) expanded figure of the globe and (B) the experimental equipment of Experiment 1

We conducted this experiment in 16:00 to 18:50 of weekdays, and in 11:00 to 17:00 of holidays (weekends and national holidays) on August 10 to September 13, 2020 (9 days in total).

The number of this experiment was 75 times (25 bumblebees × 3 times).

Experiment 2 (testing of hypothesis 2)

The position of the experiment container was same as Experiment 1.

The authors made the globe tied with thread we used in Experiment 1 go back and forth five times (eight seconds) by dropping it from 10cm in height from the bottom of the experiment container. We adjusted that bumblebees' pivot of ahead and behind be parallel with direction of the reciprocation action by revolving the experiment container. We referred the experiment which we made the reciprocation action at the left side of bumblebees as Experiment 2-1, and the right side of them as Experiment 2-2.

The days we conducted this experiment were same as Experiment 1.

We conducted this experiment 120 times (20 bumblebees × 3 times × Experiment 2-1 and Experiment 2-2).

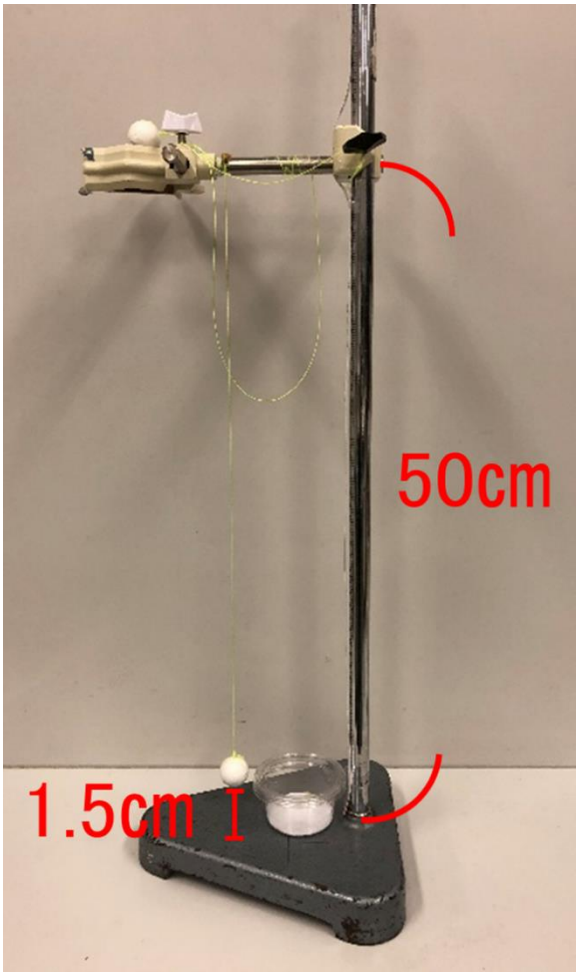

Fig.11 the experimental equipment of Experiment 2

Experiment 3 (supplement of Experiment 1)

In the Experiment 1, the vibrational stimulus when the globe collide with the experiment container and the visual stimulus when the globe fall to the above of bumblebees may have coexisted. The authors conducted this experiment to test this probability.

Experiment 3-1 (exclusion of the visual stimulus)

The authors pasted a black paper on the whole surface of the lid of the experiment container.

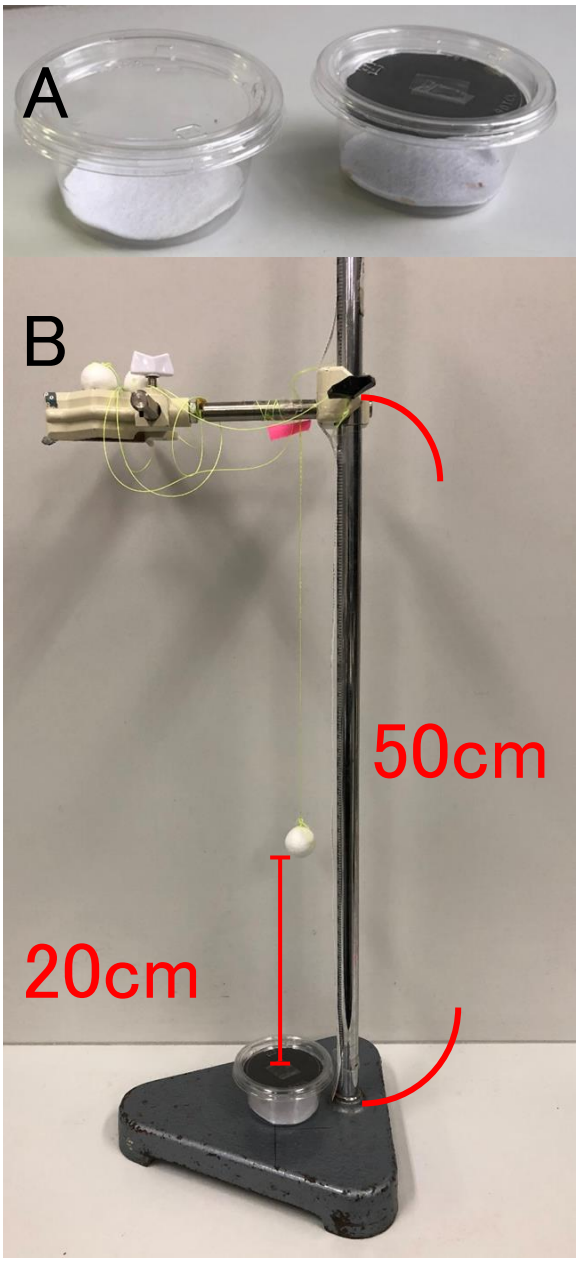

Fig.12 (A) the experiment container which we excluded the visual stimulus (right side) and (B) the experimental equipment of Experiment 3-1

The other experimental methods were same as Experiment 1.

We conducted this experiment in 16:00 to 18:50 of weekdays, and in 11:00 to 17:00 of holidays (weekends and national holidays) on September 6 to October 3, 2020 (7 days in total).

The number of this experiment was 48 times (16 bumblebees × 3 times).

Experiment 3-2 (exclusion of the vibrational stimulus)

The authors tied a thread to the globe which we adjusted that it stops before 1cm from the lid of the experiment container.

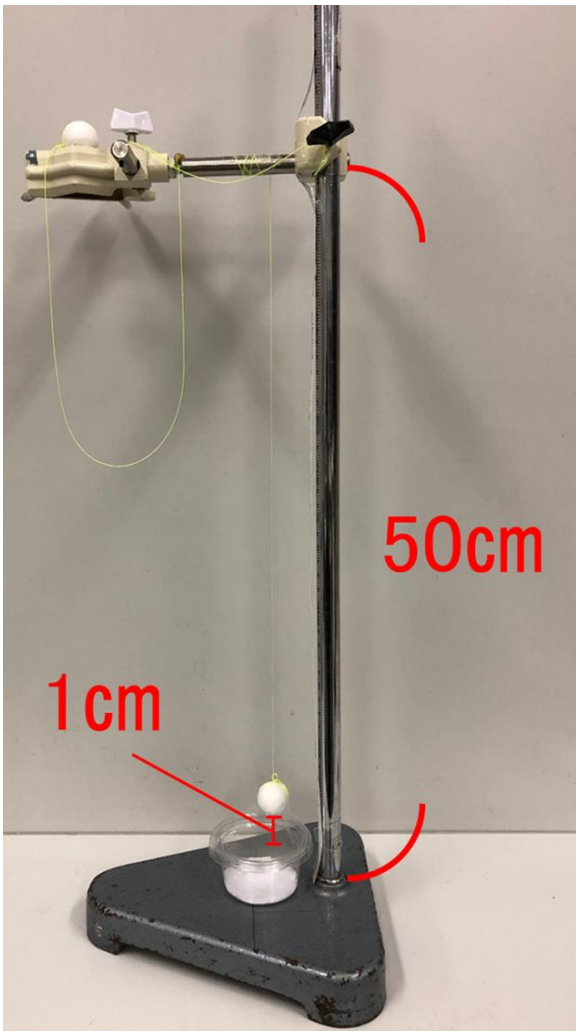

Fig.13 the experimental equipment of Experiment 3-2

The other experimental methods were same as Experiment 1.

The days we conducted this experiment were same as Experiment 3-1.

We conducted this experiment 42 times (14 bumblebees × 3 times).

Control experiments

The authors conducted the control experiments which we did not give vibrational and visual stimuli in Experiment 1, Experiment 2, and Experiment 3.

Results

Results of Experiment 1

The authors acquired the data of 75 times (25 bumblebees × 3 times) from Experiment 1. The number of the reaction was 53 times (71%) in total. Furthermore, the number of which legs caused the reaction was as follows; left middle leg was 19 times, both left middle leg and left back leg were 7 times, right middle leg was 18 times, and both right middle leg and right back leg were 9 times.

Results of Experiment 2

The authors acquired the data of 60 times (20bumblebees × 3 times) from Experiment 2-1. The number of the reaction was 17 times (28%) in total. Furthermore, only left middle leg caused the reaction.

We acquired the data of 60 times (20 bumblebees × 3 times) from Experiment 2-2. The number of the reaction was 13 times (22%) in total. Moreover, the number of which legs caused the reaction was as follows; right middle leg was 10 times, and both right middle leg and right back leg were 3 times.

Results of Experiment 3

The authors acquired the data of 48 times (16 bumblebees × 3 times) from Experiment 3-1. The number of the reaction was 10 times (21%) in total. Furthermore, the number of which legs caused the reaction was as follows; left middle leg was 7 times, and right middle leg was 3 times.

We acquired the data of 42 times (14 bumblebees × 3 times) from Experiment 3-2. The number of the reaction was 20 times (48%) in total. Moreover, the number of which legs caused the reaction was as follows; left middle leg was 6 times, both left middle leg and left back legs were 7 times, right middle leg was 3 times, and both right middle leg and right back leg were 3 times.

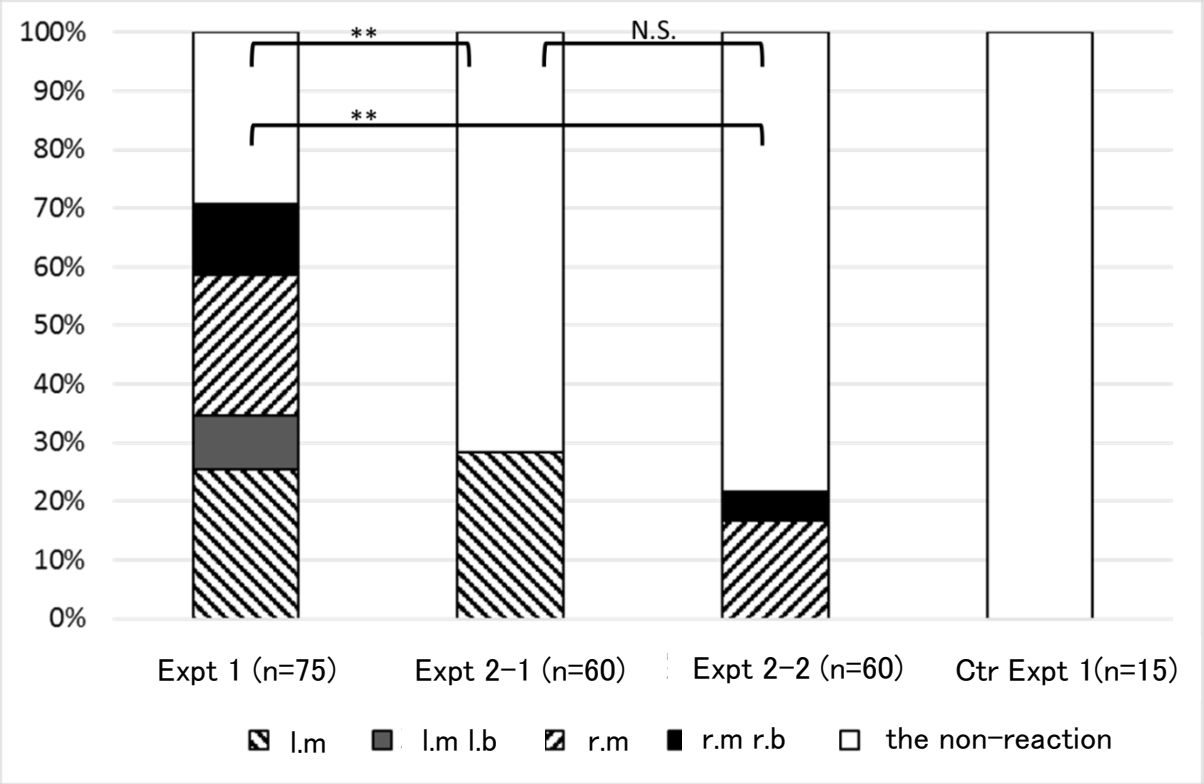

Fig.14 the comprehensive results of Experiment 1 and Experiment 2

Expt: Experiment, Ctr Expt: Control experiments, l.m: left middle leg, l.m l.b: both left middle leg and left back leg, r.m: right middle leg, r.m r.b: both right middle leg and right back leg, **: $p < 0.01$, N.S.: Not Significant

Table 1 the comprehensive results of Experiment 1 and Experiment 2

N.S.: Not Significant

	Experiment 1			Experiment 2-1			Experiment 2-2		
times	1	2	3	1	2	3	1	2	3
the reaction	17	19	17	7	5	5	6	3	4
the non-reaction	8	6	8	13	15	15	14	17	16
total	25	25	25	20	20	20	20	20	20
	N.S	N.S.		N.S	N.S.		N.S	N.S.	
	N.S.			N.S.			N.S.		

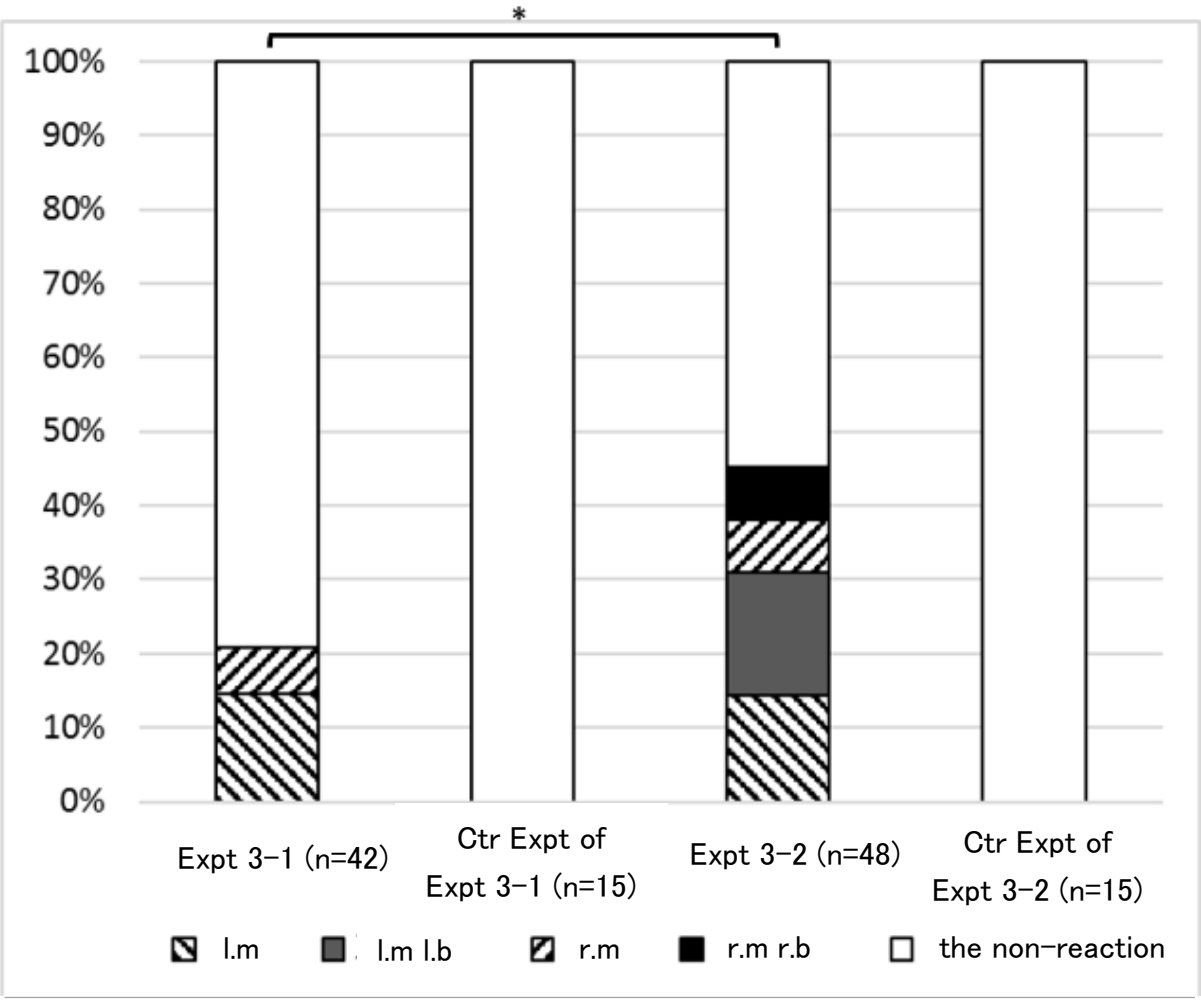

Fig.15 the comprehensive results of Experiment 3

Expt: Experiment, Ctr Expt: Control experiments, l.m: left middle leg, l.m l.b: both left middle leg and left back leg, r.m: right middle leg, r.m r.b: both right middle leg and right back leg, *: $p < 0.05$

Table 2 the comprehensive results of Experiment 3
N.S.: Not Significant.

	Experiment 3-1			Experiment 3-2		
times	1	2	3	1	2	3
the reaction	3	4	3	7	6	6
the non-reaction	13	12	13	7	8	8
total	16	16	16	14	14	14
	N.S.	N.S.		N.S.	N.S.	
	N.S.			N.S.		

Discussion

Discussion of Experiment 1 and Experiment 2

The authors conducted the same experiment three times to the same individual each in Experiment 1, Experiment 2-1, and Experiment 2-2, therefore, we considered the influence by habituation. We calculated the reaction and the non-reaction by classifying them like Table
1. Furthermore, we conducted a chi-square test. As a result, there was no significant difference between each time of the experiments. Thus, we do not consider the influence by habituation in this study, and analyze the data from Figure 14.

The proportion of the reaction was 71% in Experiment 1, 28% in Experiment 2-1, and 22% in Experiment 2-2. These results suggested that vibrational and visual stimuli are adequate for the reaction because the proportion of the reaction in all of the control experiments were 0%. Moreover, bumblebees surely raise their legs which are the same side with the reciprocating action in Experiment 2. In consequence, we estimated that the reaction is defense or warning to the coming object.

The frequency of the reaction in Experiment 1 was significantly higher than the frequency of the reaction in Experiment

2-1 and Experiment 2-2 (both $p < 0.01$ by a chi-square test).

Discussion of Experiment 3

The same as previous discussion, the authors conducted a chi-square test by calculation of Table 2. As a result, there was no significant difference between each time of the experiments. Therefore, we do not consider the influence by habituation in this study, and analyze the data from Figure 15.

The frequency of the reaction in Experiment 3-1 and Experiment 3-2 are both significantly lower than the frequency of the reaction in Experiment 1 (both $p < 0.01$ by a chi-square test), therefore, we estimated that the frequency of the reaction declined in each experiment by the exclusion the vibrational stimulus or visual stimulus.

The frequency of the reaction in Experiment 3-2 was higher than the frequency of the reaction in Experiment 3-1 ($p < 0.05$ by a chi-square test). This fact suggested that the visual stimulus is more adequate than the vibrational stimulus for the reaction.

In Experiment 1 and Experiment 3-2, both middle leg and back leg caused the reaction numerous times, therefore, we estimated that two legs were raised by large rotation of bumblebees' body by the reaction for the visual stimulus.

Conclusions

The authors reproduced the reaction in the laboratory.

The vibrational and visual stimuli are adequate to produce a reaction.

The proportion of the reaction was 0% to 71%, and the frequency of the reaction varies between each stimulus.

We revealed the four patterns of the reaction.

Both middle and back legs were significantly longer than front legs. This is our hypothesis by observation; the reaction has meaning of the defense or warning by mimicry of spiders for instance or deceiving the enemies by showing more than one individual by raising their long legs. However, more inspections are needed to estimate the ultimate factor of the reaction.

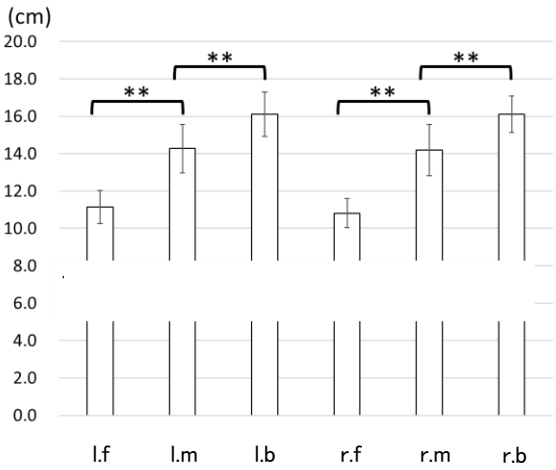

Fig.16 average of the length of the six legs and standard deviation
l.f: left front leg, l.m: left middle leg, l.b: left back leg, r.f: right front leg, r.m: right middle leg, r.b: right back leg
This graph break by white belt, however, in fact, it is continuous in all numerical value.

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