博士学位論文

Caenorhabditis elegans transfers across a gap under an electric field as dispersal behavior
（線虫 *Caenorhabditis elegans* の電場を利用した分散行動に関する研究）

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Abstract

Interactions between different animal species are a critical determinant of each species' evolution and range expansion. Chemical, visual, and mechanical interactions have been abundantly reported, but the importance of electric interactions is not well understood. Here, I report the discovery that the nematode *Caenorhabditis elegans* transfers across electric fields to achieve phoretic attachment to insects. First, I found that dauer larvae of *C. elegans* nictating on a substrate in a Petri dish moved directly to the lid through the air due to the electrostatic force from the lid. To more systematically investigate the transfer behavior, I constructed an assay system with well-controlled electric fields: the worms flew up regardless of whether a positive or negative electric field was applied, suggesting that an induced charge within the worm is related to this transfer. The mean take-off speed is 0.86 m/s, and the worm flies up under an electric field exceeding 200 kV/m. This worm transfer occurs even when the worms form a nictation column composed of up to 100 worms; I term this behavior "multi-worm transfer." These observations led us to conclude that *C. elegans* can transfer and attach to the bumblebee *Bombus terrestris*, which was charged by rubbing with flower pollen in the lab. The charge on the bumblebee was measured with a coulomb meter to be 806 pC, which was within the range of bumblebee charges and of the same order of flying insect charges observed in nature, suggesting that electrical interactions occur among different species.

Contents

1. Introduction

In this study, I report that *C. elegans* attaches to insects by passive transfer due to electrostatic attraction. There is a possibility that dispersal and the resulting distributions of very small animals have significant effects on both ecology and evolution because these submillimeter animals are at the bottom of the food chain. For small animals, especially wingless and legless animals such as worms, dispersal, and host-seeking are crucial problems because their migration processes are very slow and energetically costly. Therefore, as a dispersal strategy, smaller animals are attached to passing larger animals, such as insects or birds, to move over long distances. This kind of interaction, in which an animal uses a host animal for dispersal, is called phoresy[1,2]. Nematodes are typical examples of these small animals and exhibit characteristic behavior called "nictation," where they stand on their tail and reduce the surface energy between their body and the substrate, thus making it easier for themselves to attach to other passing objects [3–9]. The widely studied model organism *C. elegans* is known to undergo phoretic dispersal. This soil nematode is a cosmopolitan species and can be found mainly in different types of rotting plant material, such as fruits, stems, and flowers [10]. *C. elegans* does not seem to have a specific species as a dispersal vector and has been found attached to a variety of organisms, including isopods[11], snails [11], and insects [12]. Here, I found that nictating *C. elegans* transfers across a meaningful distance (a few millimeters) along an electric field from protruded substrates to electrostatically charged substrates such as the lid of a Petri dish. This observation led to the discovery that *C. elegans* can transfer across a gap (a few millimeters) to attach to anesthetized bumblebees (*Bombus terrestris*) through electrostatic interactions. I also discovered "multi-worm transfer", in which a column of nictating dauers, which consist of up to 100 worms, also transfer to an electrically charged object across a spatial gap. The experiments and finite element calculations show that their interaction is dependent on the electric field and that the bumblebee has an electrostatic charge, which is comparable with that observed in the wild. Our findings suggest the importance of electrostatic interactions for animals' phoretic interactions.

First, this chapter summarizes the ecology and life cycle of the nematode *C. elegans* and then refers to nictation, the phoretic behavior of the nematode. Next, I will introduce our discovery of the fast transfer of *C. elegans* induced by an electrostatic field and discuss the relationship between electric fields and organism behavior in nature.

In Chapter 2, I describe the observations on the fast transfer of *C. elegans* and discuss how it differs from the jumping behavior of other nematodes that have been discovered.

In Chapter 3, I discuss results from quantitative experiments on the relationship between the fast transfer of *C. elegans* and interfacial tension. In addition, I discuss results suggesting that fast transfer is caused by electrostatic interactions.

Chapter 4 introduces quantitative experimental results on the relationship between electrostatic interaction and worm's fast transfer. Based on the experimental results, the possibility that the fast

transfer of *C. elegans* is induced by an electrostatic field will be discussed.

In Chapter 4, I present qualitative experimental results on the relationship between fast transfer and electrostatic interactions in *C. elegans*, as suggested by our previous experiments.

In the following Chapter 5, I present quantitative experimental results, such as the minimum electric field intensity that causes fast transfer. In addition, I will discuss the effects of electric field sensing of *C. elegans* on fast transfer, based on comparative experiments between wild-type and mutants of *C. elegans*.

In Chapter 6, I discuss the possibility that fast transfer by electrostatic interaction can work as phoretic behavior, based on demonstrations using ground-crawling and aerial insects. Finally, I discuss the results of physical measurements and numerical analysis of the insect charges used in the demonstration.

1.1. Ecology and behavior of the nematode *Caenorhabditis elegans*

In this section, I summarize previous studies on the ecology and behavior of the nematode *C. elegans.* The ecology will focus on the life cycle and lifestyle of *C. elegans*, while the behavior will mainly describe the specific behavior called "nictation" exhibited by resistant larvae (dauer larvae), which is a special life stage of *C. elegans*. Finally, I will introduce the characteristics of leaping like the transfer of dauers discovered in our laboratory.

1.1.1. The life cycle of the nematode *C. elegans*

C. elegans is usually hermaphroditic and propagates by self-fertilization. Embryo development at 20°C is approximately 16 hours [13]. The creation of an impermeable shell post-fertilization allows the embryo to develop completely independently of the mother. The embryo grows within the mother until it reaches the 24-cell stage before being delivered. The hermaphroditic embryo hatches to 558 nuclei and becomes an L1 larva. At this time, the number of larval cells is less than the number of nuclei because the larval cells contain multinucleated fused cells. Once hatched, the larvae begin feeding and go through four life stages (L1-L4 larva) to reach adulthood. The length of the L1 larva is up to 16 hours, while the other stages are up to 12 hours. At the end of each stage is a sleep-like period called lethargus [14], during which the worms form the cuticular layer that serves as the outer skin. This lethargus period ends with the molting of the old cuticular layer, which moves the worms to the next life stage. Approximately 12 hours after the L4 larva molt, adults begin reproduction. This reproductive period is 2-3 days, during which the adults are either self-fertilized or mated with hermaphrodites and males. After completing their reproductive period, hermaphrodites live for several weeks before aging to death.

1.1.2. The dauer larvae of *C. elegans*

Depletion of prey bacteria and increased worm densities cause larvae to molt into resistant larvae (dauer larva) rather than L3 larvae. This change is initiated when L1 larvae receive signals of food depletion or increased density [15]. First, the L1 larva grows into the L2d larva, which is morphologically no different from the L2 larva. This L2d larva is like a pre-dauer larva and can revert to a normal L3 larva when the stimulus to become a dauer larva is eliminated. If further stimulation is continued, the L2d larva becomes a dauer larva in molting. Because the body of dauer larvae is completely covered by the cuticular layer, including the mouth, dauer larvae are unable to feed and body growth ceases [16]. The cuticular layer covering the entire body of dauer larvae is resistant to chemicals, and the protection provided by this layer allows dauer larvae to acquire a strong resistance to environmental stress and decay [16]. In addition, dauer larvae can survive for months without feeding because their metabolic and other functions are suspended [17]. Therefore, the dauer larvae are considered a life stage specialized for survival in the environment, and many nematodes found in the field are in the dauer stage [11,18]. This

dauer stage is terminated by the detection of food bacteria. After sensing the food, the dauer larva molts to become a L4 larva and resumes feeding and growth.

1.1.3. Nictation

It is not only their morphology and metabolism that characterize *C. elegans* in the dauer stage compared to *C. elegans* in other life stages. In terms of behavior, dauer larvae exhibit "nictation," a behavior unique to this life stage in which they stand up on their tails and swing their bodies (Figure 1.1). This behavior is thought to increase the surface area of *C. elegans* and increase the possibility of attachment to passing organisms. It is also believed that *C. elegans* is carried by these organisms, thereby facilitating its dispersal into the environment. This behavior, in which an organism (phoront) attaches itself to a host animal for dispersal, is known as phoretic behavior (or phoresy) [1,19]. Typical organisms that exhibit phoresy are animals with a limited ability to migrate, such as nematodes and mites, and it has also been observed in insects and shellfish [20–23]. Since *C. elegans* has also been found attached to isopods, snails, and insects in the field environment, it can be assumed that it uses phoresy for dispersal behavior [11,12]. In contrast to other nematodes, the phoretic relationships of *C. elegans* do not seem to be highly species-specific [18].

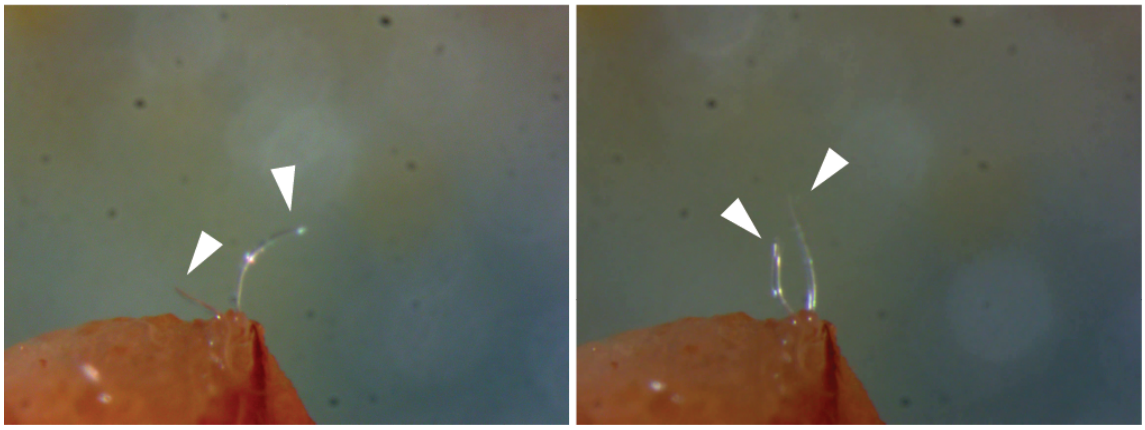

Figure 1.1 Nictation of *C. elegans* dauer larvae

1.1.4. The leaping-like transfer of *C. elegans*

C. elegans may achieve dispersal into the environment by attaching itself to other organisms to assist its low mobility. It has also been found that nictation, a standing behavior, contributes to attachment to highly mobile organisms of other species [5]. In these studies, the function of nictation was considered to be passive, such that standing up would increase the nematode's opportunity to contact other organisms passing by. Regarding the phoretic behavior of *C. elegans*, our laboratory discovered that *C. elegans* leaps at a speed of approximately 1 m/s. *Steinernema carpocapsae*, an insect parasitic nematode, is known to exhibit jumping behavior, with a jumping speed of approximately 1.134 m/s,

which is comparable to that of *C. elegans* measured in this study [3]. On the other hand, one of the obvious differences between the leap-like transfer of *C. elegans* and the leap of *S. carpocapsae* is the preliminary movement before the leap. *S. carpocapsae's* leap using a two-step process of forming and contracting a loop [3]. During loop formation, *S. carpocapsae* bends their bodies, bringing the head region into contact with the sides of the tail region. The contact surface is held by the surface tension of the water film covering the nematode. By retaining and contracting this loop, bending energy is stored in the cuticle layer of the body surface. When the loop contracts sufficiently, the surface tension breaks down, releasing enough bending energy for the *S. carpocapsae* to leap. In contrast, no such preliminary movements have been observed in the fast transfer of *C. elegans*, and the vertically immobile nematode leaps directly into the air. Remarkably, I have also observed *C. elegans* nictation columns leaping. *C. elegans* can form visible dauer columns (about 1-2 mm) by nictation of multiple individuals that twist together. Our observations confirm that these nictation columns, consisting of nearly one hundred individuals, can leap at speeds that are difficult to catch with the naked eye, as is the case with a single individual. As far as our observation, the *C. elegans* leap-like transfer is a phenomenon found only in nictating nematodes. In addition, since nictation is thought to be a behavior that contributes to the attachment of *C. elegans* to other organisms, I can expect that this leap-like transfer is also a phoretic behavior of *C. elegans*.

1.2. Phoresy

Phoresy is an interaction of a phoresy animal (phoront) with a host animal for dispersal into the environment. Typically, phoront are animals like nematodes and mites, which have limited ability to travel long distances on their own and require the aid of a highly mobile host like a fly or bee [2]. The widely used definition of Phoresy was proposed by Houck and O'Connor in 1991. According to them, phoresy is " a phenomenon in which one organism (the phoretic) receives an ecological or evolutionary advantage by migrating from the natal habitat while superficially attached to a selected interspecific host for some portion of the individual phoretic's lifetime." [19]. Since the discovery of the phoretic interaction of shells with ducks [23], this interaction has been observed in beetles and bees, nematodes and insects, and crustaceans and amphibians such as lizards and frogs [2]. These facts indicate that phoretic interactions cover a wide spectrum of the animal kingdom and are extremely diverse.

1.3. Electrostatic fields and animals' behavior

Our experiments have confirmed that fast transfer of *C. elegans* dauer larvae is frequently observed in plastic Petri dishes. I have confirmed by measurement that plastics are easily charged by contact or friction with an observer's finger or other object. Therefore, fast transfer may be induced by the electrostatic field. In Chapter 4 and the following, I will discuss the relationship between this fast transfer and electrostatic interaction, which was found in this study. The relationship between electric

fields and animal behavior has been widely studied, especially in aquatic organisms, and in recent years, the perception of electric fields by terrestrial organisms has also been investigated [24]. It has been reported that *C. elegans* is also a terrestrial organism that can sense electric fields, and the neural mechanism by which it achieves electric field perception is also known [25–27]. Based on these facts, the leap-like transfer of *C. elegans* has the possibility of phoretic behavior using the electric charge of a host animal. Animals, including insects, which are the phoretic hosts of *C. elegans*, are known to be electrically charged in their environment [24]. In Chapter 6, I will demonstrate using insects that the *C. elegans* leap-like transfer is a phoretic behavior that utilizes the electric charge of the host animal.

2. Leap-like transfer of the nematodes *C. elegans*

First, I found the nematode *C. elegans* leap-like transfer in the plastic petri dish (Figure 2.1, Video S1). This transfer is always continuing "Nictation" in which nematodes dauer larvae stand on their tail and shake heads. In all our observations, only nictating *C. elegans* showed leap-like transfer. This observational knowledge suggested that nictating behavior is necessary to transfer. This transfer is first discovered in our research and the speed of phenomena is very fast. So, the detail of behavior is not observed nor how nematodes achieve such a fast transfer. Then I first tried to observe *C. elegans* leap-like transfer in detail by using the high-speed camera.

2.1. Methods

The nematode used in the experiments was *C. elegans* Bristol strain N2, which was cultured and maintained by standard methods using Nematode Growth Medium (NGM) agar plates with *Escherichia coli* OP50 [28]. As noted above, only dauer larvae can nictate and leap. To obtain the dauer larvae, *C. elegans* were cultured using the Dog Food Method (DFM) reported in a previous study [29,30]. Briefly, a mixture of 180 g powdered dog food (Keiyo), 3.0 g agar (Inakanten S-7, Ina industry), and 300 ml reverse osmosis water was autoclaved and transferred to a 60 mm petri dish, in which the dish was filled with the mixture; to flatten the surface, the lid was placed in contact with the mixture. After storing it at 4 °C for 24 hours to coagulate agarose, the mixture was cut into a cube (approximately 0.5×0.5×0.5 mm) and placed on the center of the NGM agar plate with *E. coli* OP50. The worms cultured on the NGM agar plate were transferred to the NGM agar plate with the DFA and incubated at 20 °C for 12–16 days. In this process, worms become dauer larvae, and numerous larvae were collected on the lid of the NGM agar plate.

After confirming that many dauer larvae were nictating on DFA cubes, I observed *C. elegans* leap-like transfer Images of dauer larvae leap-like transfer viewed from above were obtained by observing dauer larvae nictating on DFA on the NGM plate using a CMOS camera (11 fps, ORCA-spark, Hamamatsu photonics) and a high-speed camera (40,000 fps, FASTCAM Mini AX200, Photron).

2.2. Results

Figure 2.2 shows a snapshot of the leap-like transfer of *C. elegans* obtained by a high-speed camera. This series of images showed that *C. elegans* hardly changes its posture; the shape of the body during the leap is nearly straight (Figure 2.2A, Video S2). In addition, leaping behavior was observed with one nematode lifting multiple nematodes (Figure 2.2B, C and Video S3, S4). Such a leap in a nictation column state which consists of multiple individuals will be referred to as a multi-worm transfer and a leap by a single nematode will be referred to as a single-worm transfer. In this multi-worm leap, as in the single-worm transfer, the body posture of the leaping nematode hardly changed at all. In addition,

no significant movement could be seen in the tail of the nematode, the only part of the body that contact with the substrate before leaping. These behavioral characteristics are quite different from that of the leap of *Steinernema carpocapsae* [3]. This insect parasitic nematode bends its body before leaping to create a loop structure and store interfacial energy at the contact surface. As shown in Figure 2.2A-E, *C. elegans* did not exhibit any of these preliminary movements in its leaping. This observation suggests that the leaping behavior of *C. elegans* dauer larvae is achieved by a different mechanism than the leaping using the surface tension of *S. carpocapsae*.

Observations using a high-speed camera revealed that the leap-like transfer of *C. elegans* has unique features that differ from the previously reported leaping behavior of *S. carpocapsae*. I next measured the leaping velocities of single and multi-worm transfer under different interfacial tension conditions to obtain quantitative data on this leap-like transfer.

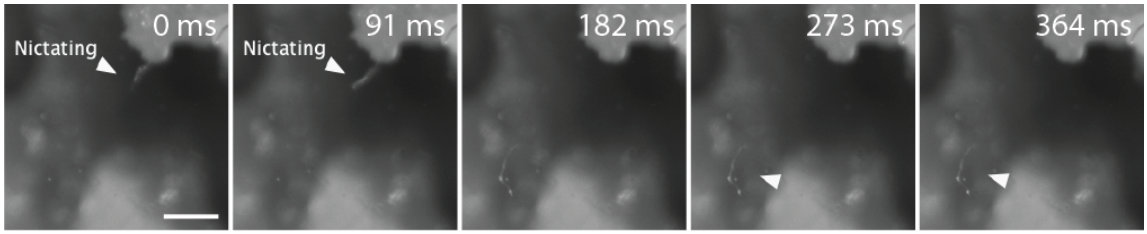

Figure 2.1 Fast transfer of *C. elegans* daure larva in a Petri dish. Scale bar, 0.5 mm. See also Video S1.

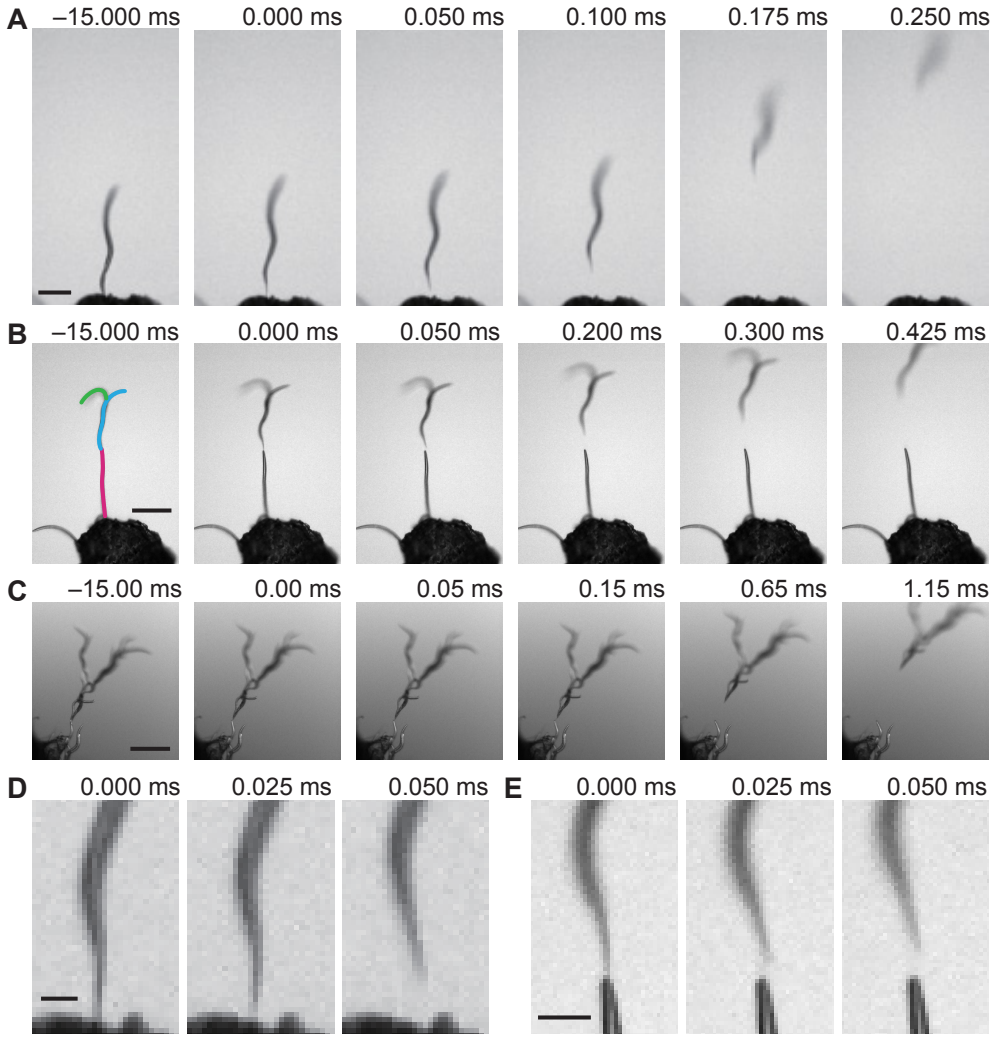

Figure 2.2 Leap-like transfer of *C. elegans* dauer larvae on the DFA in a Petri dish. (A) Transfer of a single dauer larva from DFA. Scale bar, 100 µm. See also Video S2. **(B)** Transfer of two dauer larvae (blue and green) that are attached together from the tip of a nictating nematode (magenta). Scale bar, 200 µm. See also Video S3. **(C)** Leap-like transfer of a nictation column that consists of more than 20 dauer larvae from the tip of a nictating nematode. Scale bar, 400 µm. See also Video S4. **(D, E)** Snapshots of the movement of the tail of a dauer larva during take-off. Scale bars, 20 µm, and 40 µm, respectively.

3. Measurement of leaping speed in single and multi-worm transfer

C. elegans is often found in humid environments in the field. In the laboratory environment, *C. elegans* is cultured on agar medium, so the body surface of the nematode is always wet. This suggests that *C. elegans* needs to overcome the interfacial tension of the water received from the ground surface to transfer. This is consistent with the fact that all observed nematode leap-like transfers occur following nictation. In this section, I describe the experiments I conducted to examine the effect of the surface tension of the water on the leap-like transfer.

In previous observation systems, I have observed the leap-like transfer of dauer larvae nictating on DFA. These larvae are those growing on agar medium that has been cultured for approximately two weeks. Therefore, they exist in an environment that contains a variety of impurities, including chemicals contained in the NGM and metabolic products produced by the culture. To clarify the effect of surface tension on leap-like transfer, it is necessary to observe the transfer of dauer larvae under conditions in which these impurities have been removed. Therefore, I constructed a new system that allows observation of nematode leap-like transfer under conditions in which the composition of the external environment is controlled and observed the nematode leaping speed in systems with and without detergent added.

3.1. Methods

3.1.1. Preparation of nematode suspension

C. elegans dauer larvae were cultured in large numbers in the DFM described in Methods (2.1 Methods). Since this incubation causes a large number of dauer larvae to collect on the petri dish lid (Figure 3.1), these were collected in 1 mL of ultrapure water. The nematode suspension was left to stand for several minutes, and the precipitated nematodes were collected and transferred to 1 mL of fresh ultrapure water. This wash process was repeated three times. In experiments with detergent added, dauer larvae were collected and washed in 0.02% (v/v) Triton X-100 instead of ultrapure water to obtain suspensions.

3.1.2. Construction of observation system

From previous observations of leap-like transfer, it was observed that dauer larvae frequently nictate on sharp structures (e.g., DFA and the edges of agar media). In this experiment, nylon mesh was used as a substrate to mimic the structure that facilitates nictation in these nematodes. A 9 cm petri dish was filled with 1 % agar in a half-moon shape. Nylon mesh with an aperture of 18.0 μm, cut into 7 mm long and 4 mm wide by a cutting machine (CE6000-40 Plus, GRAPHTEC), was inserted vertically into the petri dish and used as a substrate for leaping (Figure 3.2).

3.1.3. Observation of the leap-like transfer

Forty μL of the nematode suspension prepared by Method A was dropped onto the root of a nylon mesh. Excess water was removed with a Kimwipe and placed for a few minutes to allow dauer larvae

to begin nictation on the nylon mesh. An image of the nematode's transfer, viewed from the side, was obtained by a high-speed camera (FASTCAM Mini AX50, Photron) with a macro lens (3554B001, CANON) at 10,000 fps. Filming was done for single-worm transfers as well as for multi-worm transfers by waiting for nictation columns to form, and 1-4 nematode transfers were filmed.

3.1.4. Calculation of leaping speed by image analysis

Two-dimensional coordinate data of the leaping nematodes were extracted in chronological order using Fiji (ver. 2.0.0-rc-69/1.52p, Schindelin et al., 2012). The position of the nematode coordinates was determined by visually plotting the tail (Figure 3.3, Video S5). Visual positioning was performed for the three frames before and after the leap-like transfer as shown in Figure 3.3. The first three frames (0.2 ms) of the image were used for the velocity measurement because the nematode would be out of focus over time. The leaping speed was calculated using Numpy (ver. 1.17.2)[32] for 0.2 ms of the nematode's positional data.

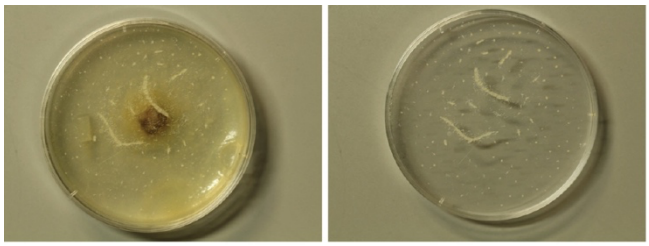

Figure 3.1 *C. elegans* dauer larvae cultured by DFM. The photo on the left shows dauer larvae cultured on NGM and DFA gathering on the lid of a polystyrene petri dish. In the photo on the right, only the lid of the petri dish was photographed.

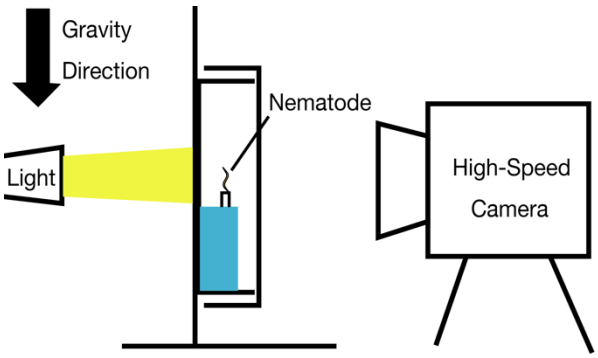

Figure 3.2 Experimental Setup for leaping speed measurement.

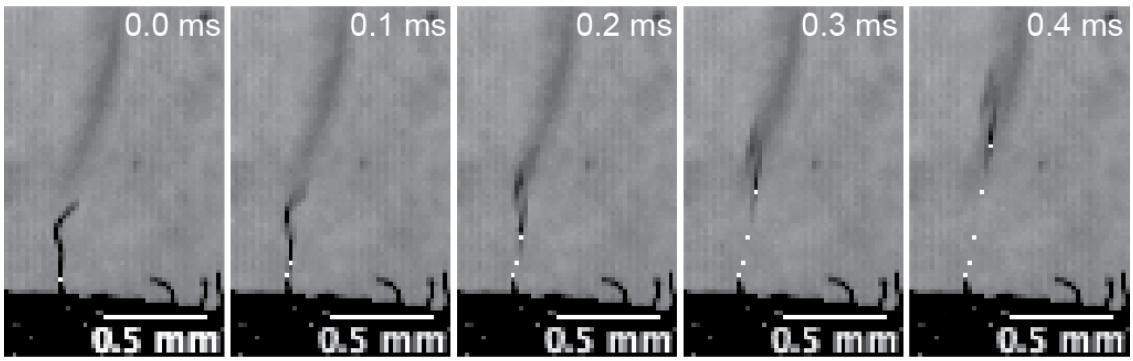

Figure 3.3 *C. elegans* dauer larvae transfer from nylon mesh. The white dot on the tail is the position of the dauer plotted visually. Scale bar, 0.5 mm. See also Video S5

3.2. Results

3.2.1. Effects of nematode number and surface tension on leaping speed.

Figure 3.4 shows snapshots of single and multi-worm transfers taken with a high-speed camera. Figure 3.5 also shows the relationship between the number of nematodes and leaping speed in the system without and with detergent. Figure 3.5A shows that the leaping speed of dauer is 1.08 ± 0.38 m/s (median ± SD). The leaping speeds of two and three dauers were 0.70 ± 0.31 m/s (median ± SD) and 0.72 ± 0.17 m/s (median ± SD), respectively, suggesting that the leaping speed tended to decrease as the number of nematodes increased. This trend was also observed for systems in which surface tension was lowered by detergents. In addition, it was confirmed that the addition of detergent increased the leaping speed (Figure 3.5B). The leaping speed was 1.76 ± 0.35 m/s (median ± SD) in single worm leaps, which is about 1.6 times higher than under conditions in which the surface tension was not reduced (Figure 3.5A). Leaping speeds in two and three nematodes were also increased, to 1.23 ± 0.43 m/s and 0.98 ± 0.30 m/s, respectively (0.70 ± 0.31 m/s in two nematodes and 0.72 ± 0.17 m/s in three nematodes under the no addition condition).

3.2.2. Involvement of electrostatic forces in leap-like transfer

Using the constructed observation system, the number of nematodes and the surface tension were found to affect the leaping speed. Because the shape of the leaping nematode was out of focus, the leaping speed was calculated using the initial three images which were taken 0.2 ms after the nematode left the substrate. Looking at the longer time leap trajectories as a qualitative observation, it was found that their trajectories were curved rather than straight (Figure 3.6, Video S6). In addition, the time evolution of leaping speed in some samples showed the acceleration of the nematodes (Figure 3.3 and Figure 3.7). These results suggest that some external force is acting on the nematode during leaping.

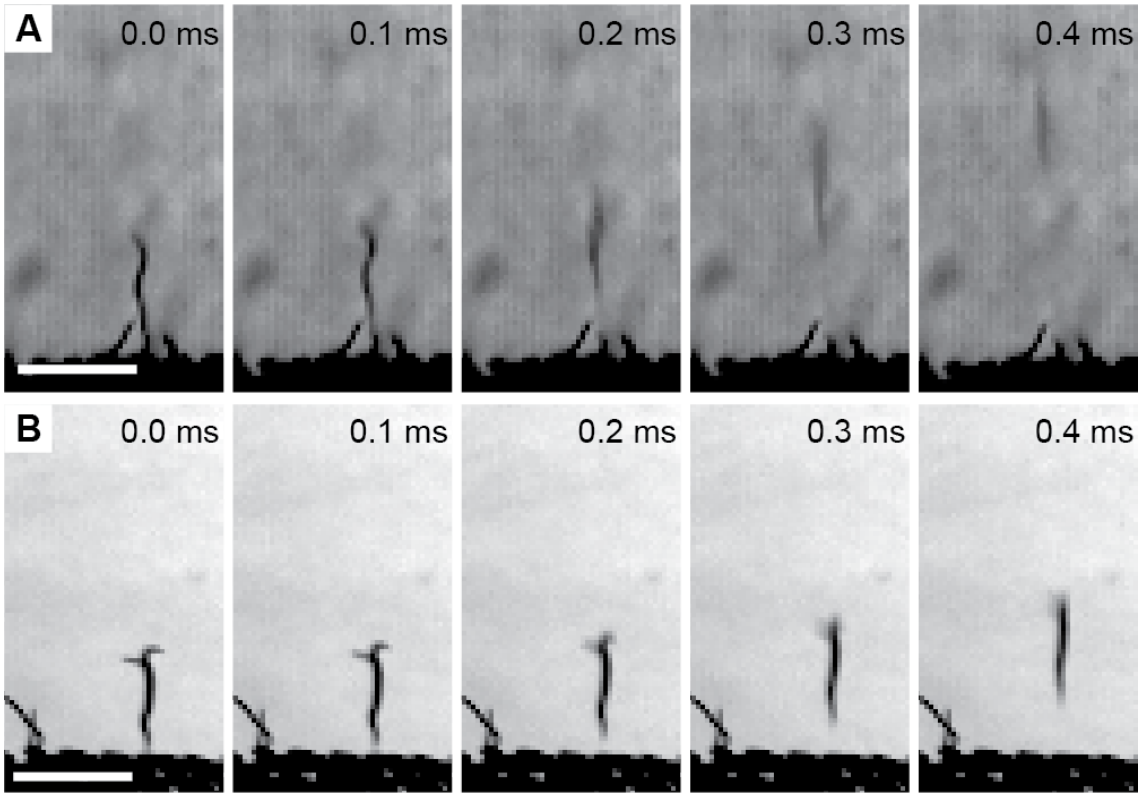

Figure 3.4 *C. elegans* dauer larvae transfer from nylon mesh. (A) Single-worm transfer. **(B)** Multi-worm transfer. Scale bar, 0.5 mm.

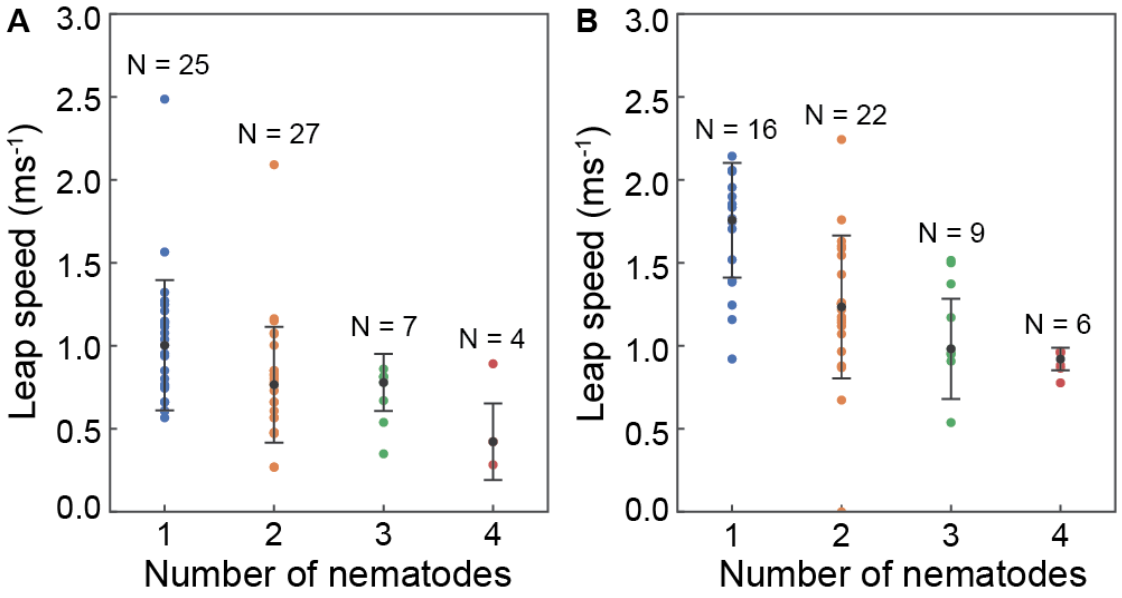

Figure 3.5 Effect of surface tension on leaping speed of *C. elegans* dauer larvae. (A) Leaping speed of dauer larvae under conditions without detergent addition. Black dots represent the median; error bars represent the SD. **(B)** Leaping speed of dauer larvae under detergent-added conditions. Black dots represent the median; error bars represent the SD.

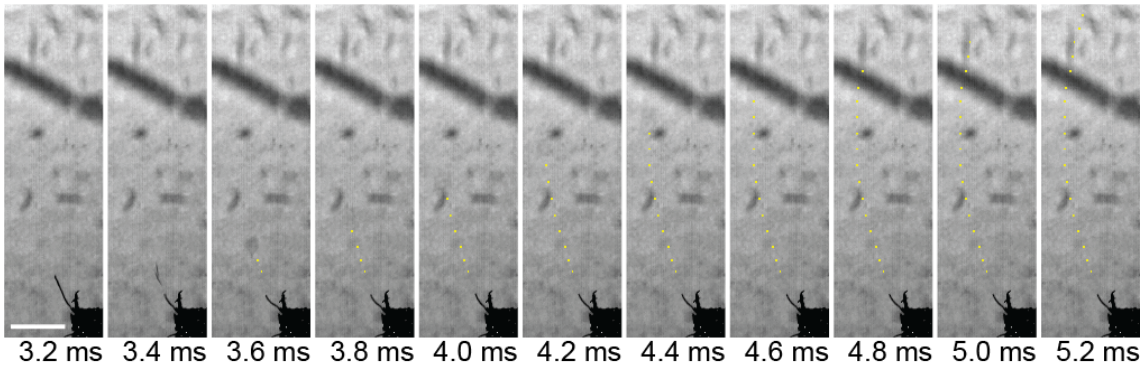

Figure 3.6 Leaping dauer larva with curved trajectories. The vertical axis is the z-axis. Scale bar, 1.0 mm. See also Video S6.

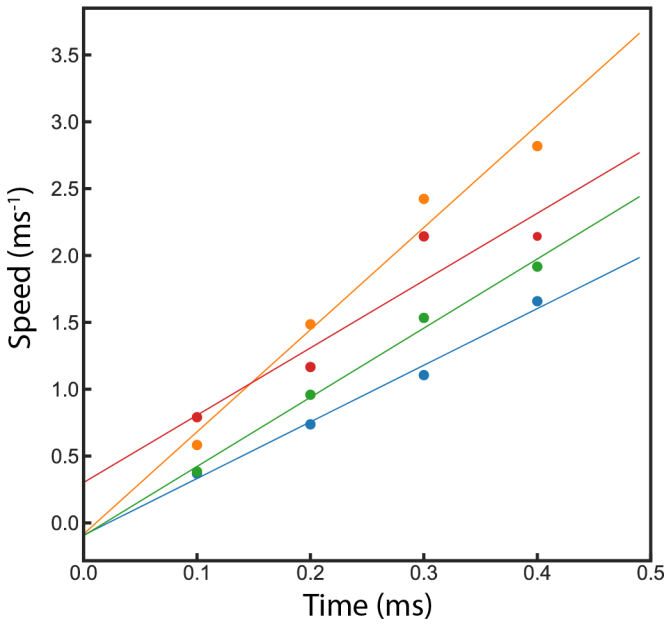

Figure 3.7 Time evolution of nematode leaping speed. Plots are color-coded for each sample (orange, red, green, and blue). Regression lines were calculated by the least squares method.

3.3. Conclusion

I attempted to characterize the dynamics of the leap-like transfer of *C. elegans* dauer larvae by measuring their speed under various conditions. As a result, the leaping speed of one nematode was calculated to be 1.08 ± 0.38 m/s (median ± SD). The addition of a detergent to reduce the surface tension acting on the nematode increased the nematode's leaping speed. These results suggest that *C. elegans* transfers by overcoming the surface tension at the interface with the ground surface. Furthermore, both experiments at these two different surface tensions showed a decrease in speed as the number of nematodes increased. Considering that only one nematode is leaping in multi-worm transfers, I can assume that the leaping speed is dependent on the mass of the nematode.

Through this study, I found that the leaping speed of *C. elegans* dauer larvae is 1.08 ± 0.38 m/s (median ± SD). The reported leaping speed of Steinernema spp. (*S. carpocapsae*) was calculated to be 1.134 m/s [3], which is almost equal to that of *C. elegans*. However, the behavioral sequence before leaping is quite different. *S. carpocapsae* is known to make a preliminary movement such as creating a loop structure with its body, and *S. carpocapsae* jumps by releasing the interfacial energy stored in this loop structure [3]. It takes approximately one second for the nematode to leap from the start of the formation of this loop structure [3]. On the other hand, the leap-like transfer of *C. elegans* does not include any preliminary movements like those performed by *S. carpocapsae*. As shown in Figure 2.2 and Figure 3.4, *C. elegans* dauer larvae leap in a straight posture, hardly moving even their tails. Thus, it appears that *C. elegans* is leaping without using energy generated by itself, such as bending energy stored in the cuticle layer.

By measuring the leaping speed of *C. elegans*, I found that the leaping *C. elegans* follows a curved trajectory. In addition, image analysis revealed that the leaping *C. elegans* accelerates in the air. All the leap-like transfers observed in this experiment occurred in a plastic petri dish. This plastic petri dish is made of polystyrene and is easily charged by contact or friction with fingers or other objects. Therefore, it can be inferred that the curvature of the leaping trajectory and the acceleration in the air observed in this experiment is due to the electrostatic field resulting from the charging of the plastic petri dish. In the next chapter, qualitative experiments to confirm the effect of the electrostatic field are described, followed by a discussion of the results of the experiments.

4. Observation of fast transfer under conditions of low electrostatic field.

From the experiments in the previous chapter, I have shown that the leaping *C. elegans* accelerates in the air. I also confirmed that the trajectory of the leaping is not a straight line but a curve. These observations suggest that electrostatic interactions might affect the fast transfer of *C. elegans* as an external force. To confirm this, I examined whether *C. elegans* dauer larvae perform the fast transfer in glass Petri dishes and qualitatively confirmed the effect of the electrostatic field on leaping.

4.1. Methods

To qualitatively confirm the effect of an electrostatic field on the leap-like transfer of *C. elegans*, I confined the nematodes under less electrostatic conditions and observed them for a long time to see if the leap-like transfer occurred. First, dauer larvae cultured in DFM were placed on agar with dog food agar in a glass petri dish, which generates less electrostatic charges than the polystyrene petri dish. The dog food agar was stuck with the same nylon mesh that had been used previously and used as a base for leaping. A silicone sheet was attached to a glass shale surrounding the agar and filled with glycerol around the outside to prevent the nematode from crawling to the lid (Figure 4.1). The petri dish was lidded with a glass plate and set on a stereomicroscope (Olympus SZX16, Olympus), and a CMOS camera (ORCA-spark, HAMAMATSU Photonics) was used to capture images to confirm whether the leaping nematode stuck to the glass plate. As a control experiment, the same imaging was conducted with a lidded polystyrene plate, which tends to be easily electrically charged. Images were taken at 0.1 fps for 7 hours (glass) and 3 hours (polystyrene), respectively, in a room adjusted to 20°C.

4.2. Results

Under less electrostatic conditions (a glass plate lidded), *C. elegans* dauer larvae did not transfer at all during the 7 hours (Figure 4.2). After the imaging was completed, the nematodes were observed, and it was confirmed that several nematodes were nictated on the nylon mesh (Figure 4.3). Therefore, it is unlikely that none of the nematodes jumped because they were not able to nictate. In addition, no nematodes were found to have transferred during the three hours of observation under conditions in which the lid was covered with an easily electrified polystyrene plate (Figure 4.4). When the polystyrene plate was lightly rubbed with a finger to induce electrification, the nematodes nictating on the nylon mesh immediately transferred and were observed to stick to the lid (Figure 4.5).

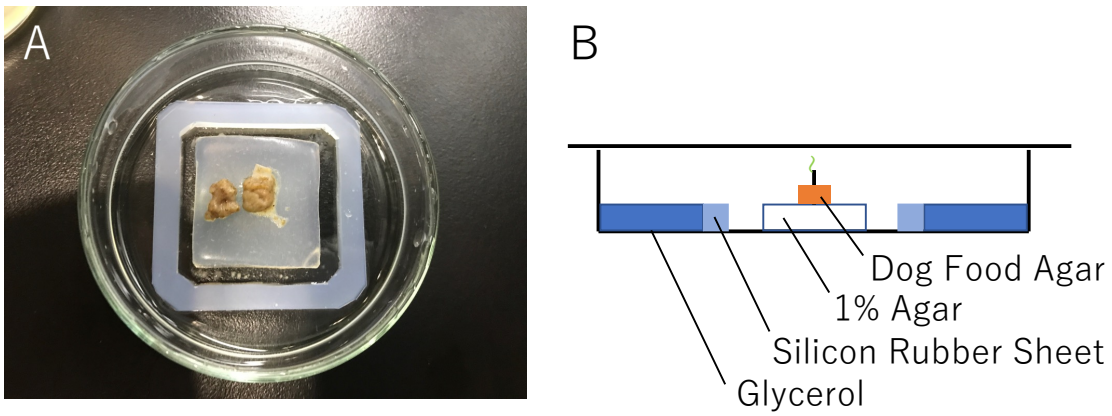

Figure 4.1 Experimental setup. (A) Photograph taken from above. (B) Schematic view from the side. To prevent the nematodes from crawling around in the glass petri dish to the lid, a moat was made with a silicon sheet and glycerol was poured into the moat. DFA was placed on 1% agar to prevent the nematodes from drying out.

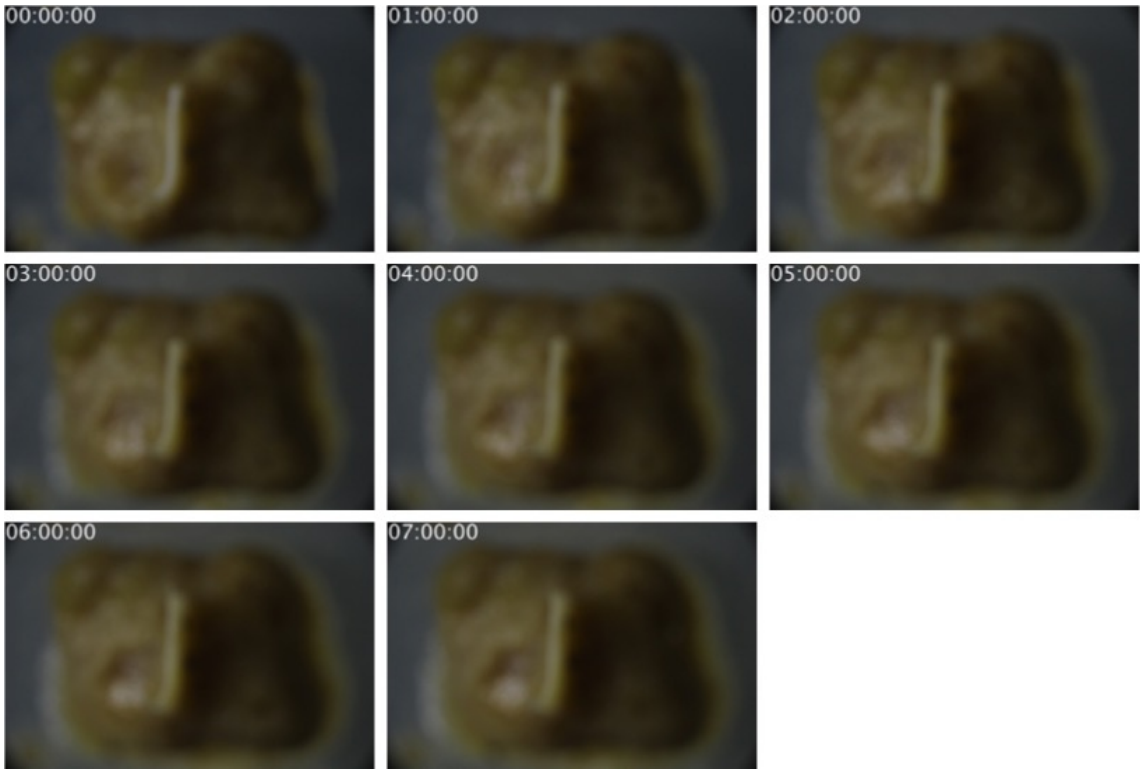

Figure 4.2 Results of observation when a glass plate is used as a lid. The brown square is the dog food agar and the white one in the middle is the nylon mesh that serves as the base of the nictation.

Figure 4.3 Nictation of *C. elegans* dauer larvae on nylon mesh.

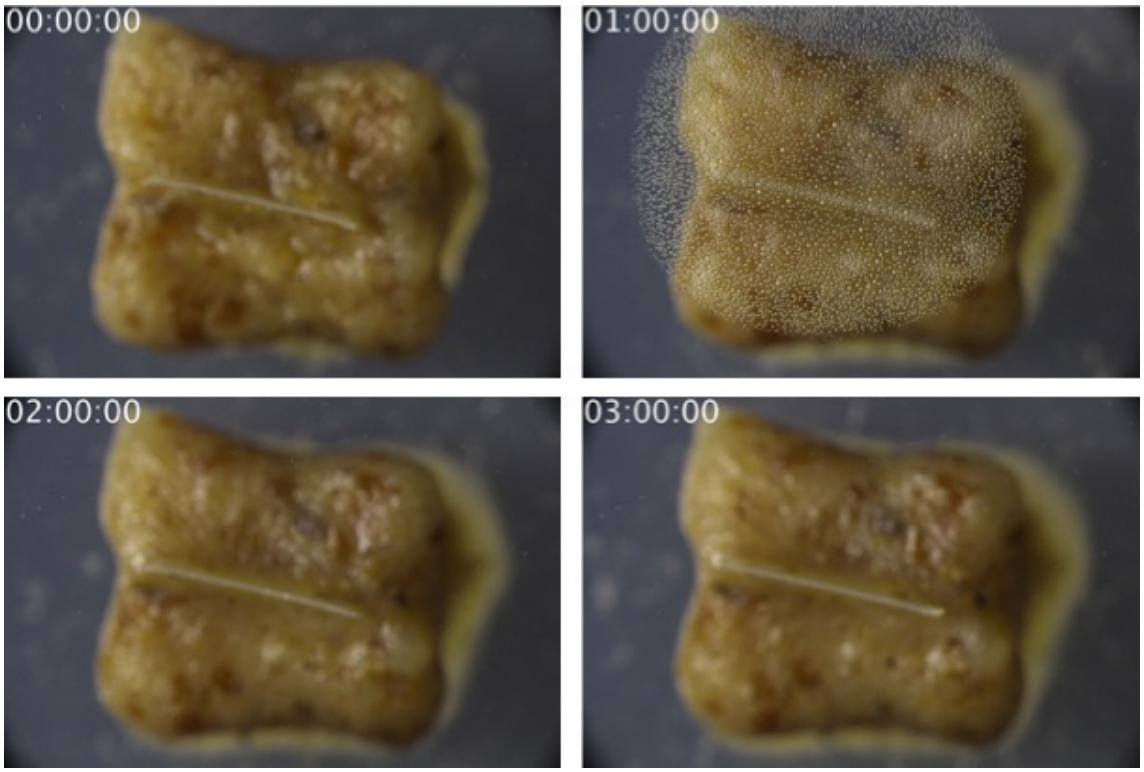

Figure 4.4 Observations made when the lid is made of a polystyrene plate, which is easily charged. The brown square is the dog food agar and the white one in the middle is the nylon mesh that serves as the base of the nictation.

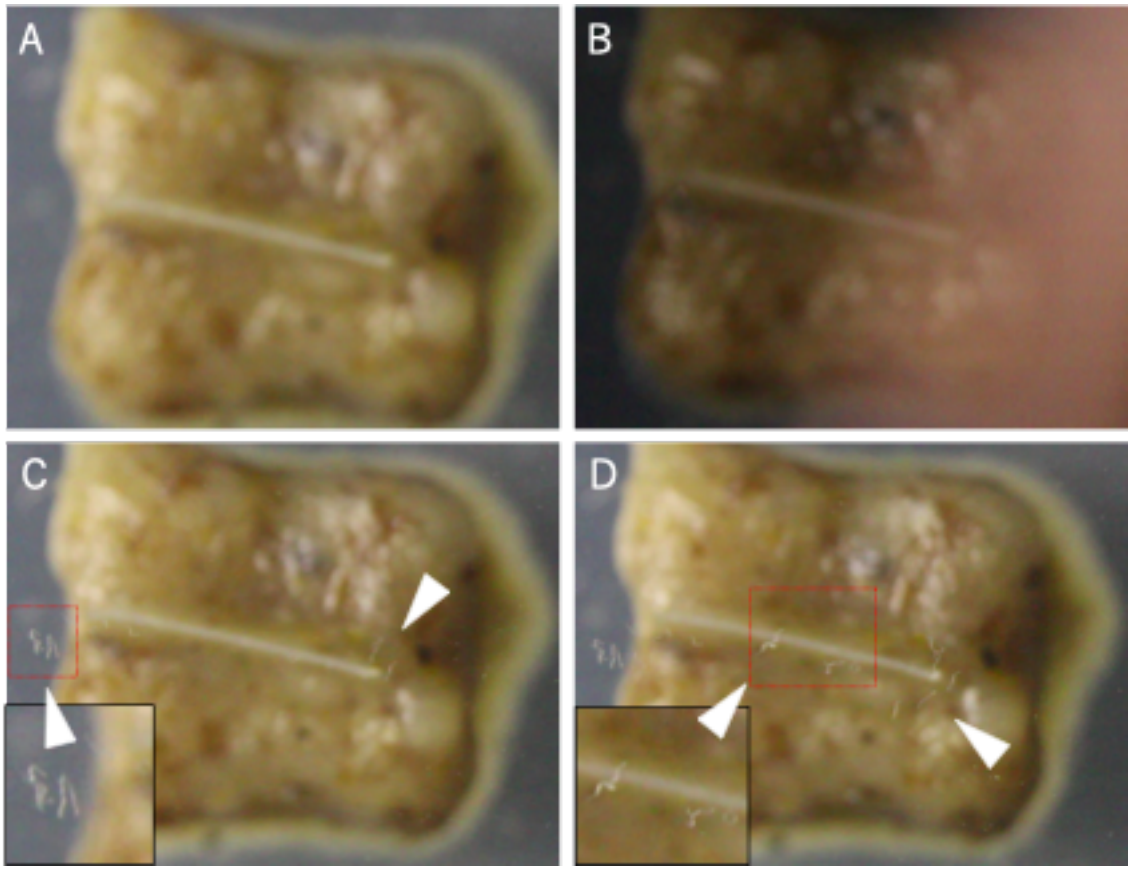

Figure 4.5 *C. elegans* dauer larvae transfer after finger rubbing. (A) After 3 hours of imaging. (B) Rubbing a polystyrene plate with their fingers. (C, D) Dauer larvae transfer after rubbing with fingers.

4.3. Conclusion.

From the results of a series of examinations, the effect of the Coulomb force due to the electrostatic field was suggested as a candidate mechanism that enables this fast transfer without any preliminary action. To confirm this, I first determined whether the nematodes would show transfer in a glass petri dish rather than the polystyrene petri dish I have been using. Glass is less electrically charged than polystyrene, so if the fast transfer is due to an electrostatic field, nematodes would not transfer much in the glass petri dish. The results of glass petri dish experiments showed that none of the nematodes transferred during the 7-hour observation. In contrast, observation in a polystyrene petri dish showed that nematodes that had not transferred for 3 hours immediately transferred to the lid when the lid was rubbed with a finger. These facts strongly support the hypothesis that *C. elegans* is leaping by using electrostatic fields. This qualitative observation can thus suggest that the transfer of *C. elegans* dauer larvae is related to the ambient electric field. In the next chapter, I will discuss the relationship between dauer larvae leaping behavior and electrostatic fields by examining quantitative experiments.

5. Leap-like transfer of *C. elegans* induced by an electrostatic field.

The purpose of this chapter is to conduct quantitative experiments on the relationship between nematode leap-like transfer and electrostatic fields, as suggested by previous experiments. Various studies have shown that insects that have a phoretic relationship with nematodes are charged in nature [33]. Therefore, it can be expected that the leap-like transfer I discovered in this study is one aspect of attachment behavior to other organisms using an electrostatic field. To confirm this, I first quantitatively measured the electric field responsiveness of leap-like transfer.

5.1. Methods

Making SU-8 photoresist and PDMS molds.

To establish nictating behavior, I used polydimethylsiloxane (PDMS)-based microfabrication methods to prepare masters. These microfabrication structures are similar to those in a previous study[5]. In detail, the acetone-washed silicon wafers were baked at 150 °C for 3 minutes and then left for 5 minutes to return to room temperature. A few drops of HMDS were applied onto the silicon wafers and spin-coated at 1000 rpm for 10 sec. SU-8 2500 negative photoresist (MICROCHEM) was spun onto a silicon substrate at a spin-coating speed of 500 rpm for 10 sec and 3000 rpm for 27 sec to obtain a uniform thickness of 25 μm. Silicon wafers coated with SU-8 were soft-baked at 65°C for 5 min, then at 95°C for 1 hour. The photomask and baked silicon wafer were fixed in place with a mask aligner (MA-20, Mikasa) and exposed to UV for 40 sec. The exposed silicon wafers were baked at 65°C for 2 min and 95°C for 6 min and then developed in PGMEA. After confirming that the structures were formed by development, the obtained SU-8 master mold was rinsed with IPA.

The curing agent and PDMS prepolymer were mixed in a 1:10 weight ratio and degassed for 1 hour to remove trapped air bubbles. A trimethylchlorosilane (TMCS) vapor treatment was applied to the molds to facilitate the demolding of the PDMS from the SU-8 mold. Briefly, the treatment was conducted in a glass petri dish in which the TMCS solution was dropped onto the SU-8 mold surface. After about 2 minutes of this vapor treatment, the degassed PDMS mixture was poured onto the SU-8 mold. At this time, the petri dish containing the PDMS was lidded with a glass flat plate to prevent air bubbles from forming, so that the bottom of the resulting PDMS mold would be flat. This PDMS mixture was cured by baking at 80°C for 1 hour. After peeling the resulting PDMS from the mold, I confirmed whether the desired structures were formed.

Preparation of the microdirt substrate.

The structure consists of a square array of micro-posts; each post is 25 μm high, 50 μm in diameter, and equally spaced at 50 μm intervals. The photolithographically prepared masters (SU-8 2500, MICROCHEM) were replicated with PDMS (Silgard 184, Dow Corning) and used as templates. Four percent agar (Inakanten S-7, Ina industry) was poured onto the PDMS mold. After removing air

bubbles, the agar was left to cool at 4 °C. I named this 4% agar with a square array of posts the microdirt substrate (MDS). The MDS was soaked in 1 L of ultrapure water for 6 hours to remove ions and additionally soaked in 3 L of ultrapure water for 12 hours. After the MDS was removed from ultrapure water, extra water was removed and the MDS was dried at 37 °C for 1 hour before use.

Cultivation of *C. elegnas* mutant dauer larvae.

C. elegans mutants *che-2 (e1033)*, *osm-5 (p813)*, and *tax-6 (p678)*, defective in electric field stimuli [25–27], were cultured as in N2 for dauer larvae (see Method 2.1).

Preparation of the worm suspension.

C. elegans dauer larvae were collected in 1 mL ultrapure water as in previous Methods (2. Preparation of the nematodes suspension). The obtained suspension was added to 30 mL ultrapure water and allowed to stand for 30 minutes to settle the dauer larvae. The dauer larvae were collected with l mL solution and added to 30 mL ultrapure water again. This treatment was performed three times. After washing, the concentration of the worm suspension was adjusted to 30 worms/μL by adding ultrapure water.

Behavioral observations of dauer larvae under an applied voltage.

To evaluate the effect of an electric field on leap-like transfer, I applied an electric field to dauer larvae and measured their leaping activity. Fifty microliters of washed worm suspension were placed on the MDS, and extra water was removed (Figure 5.1). Then, the MDS was dried for approximately 15-20 minutes at room temperature and set between two parallel indium tin oxide (ITO) glass slides (15 Ω cm^{-2}, Mitsuru Optical Laboratory). The distance between the upper glass and the MDS (d) was adjusted to be approximately 4.4 mm (Figure 5.2). The voltage was applied to the upper ITO glass electrode by an amplifier (Model-610E COR-A-TROL, Trek) with a function generator (FGX-2200, TEXIO), while the lower ITO glass was grounded. For each experiment, the distance (d) between the upper electrode and the MDS was measured, and an appropriate voltage was applied to achieve the target electric field intensity from the relationship equation between the potential (V) and electric field (E) (dE=V). The entire setup was situated on the stage of an Olympus SZX16 microscope. After applying an electric field for 5 minutes, images of the surface of the upper ITO glass on which the leaping dauer larvae were attached were obtained by a CMOS camera (ORCA-spark, Hamamatsu photonics). The number of dauer larvae was counted by detecting them by intensity and size after background removal on the obtained images using Fiji (ver. 2.0.0-rc-69/1.52p)[31].

I used this electric field generator to observe the leap-like transfer of individual dauer larvae from the side. Images were obtained at 20,000 fps by using a high-speed camera (FASTCAM Mini AX50, Photron) with a macro lens (3554B001, CANON). The position of the dauer larva extracted by the

image intensity exhibited the velocity and acceleration of the leap using NumPy (ver. 1.17.2)[32].

5.2. Results

5.2.1. Relationship between electric field intensity and leaping rate.

Figure 5.3 shows images of ITO glass before (0 min) and after (5 min) voltage application. The results show that *C. elegans* dauer larvae leap and continue to attach to the surface of the ITO glass when an electrostatic field is applied. It was also confirmed that nematodes attached to ITO glass do not disappear by falling or other means. Therefore, it is possible to measure the number of dauer larvae leaping per unit time for each electric field intensity. So next, I confirmed the leaping rate of dauer larvae for each electric field intensity (E). The results showed that the minimum E at which *C. elegans* dauer larvae leap is 200 kV/m and that the higher the value of E, the more dauer larvae leap (Figure 5.4). In addition, no leaping was observed when E = 0 kV/m (electric field off). Dauers leaped whether the electric field was positive or negative, and the leaping behavior of the worms was almost the same for both positive and negative voltages if |E| is the same (Figure 5.5). These results strongly suggest that electrostatic fields are involved in the leaping behavior of *C. elegans* dauer larvae.

To get some insight into the relationship between this fast transfer and the sensitivity of nematode to the electric field [36–38], I measured the leaping frequency of mutants of *C. elegans*. First, I attempted to get *che-2(e1033)*, *osm-5(p813)*, *tax-6(p678)* mutants dauer larvae. However, it was unable to obtain enough dauer larvae of the *che-2(e1033)* and *osm-5(p813)* mutants with the dog food culture method I used (see Method 2.1). Therefore, only *tax-6(p678)* mutants were used in the leap frequency measurement experiments. The results showed that the number of leaping nematodes per minute under an electric field of 1000 kV/m intensity was 58.13 ± 16.03 worms/min (mean ± SD, N = 8) for N2 (wild type) and 3.38 ± 3.62 worms/min (mean ± SD, N = 8) for *tax-6(p678)* mutants (Figure 5.4). I also confirmed that *tax-6(p678)* mutants exhibit nictation on the substrate (MDS) and that nictation is not inhibited by the application of an electric field. These results imply that the electric field sensitivity of the nematode may be related to the leap-like transfer under electric fields.

5.2.2. Observation and quantification of leap-like transfer of *C. elegans* dauer larvae in the presence of an electric field.

Next, I observed the leap-like transfer of *C. elegans* dauer larvae using the same imaging system. Figure 5.6 and Video S7 show the leaping behavior of dauer larvae observed at each electric field intensity. At both electric field intensities, dauer larvae did not exhibit any kind of preliminary movement and were observed leaping in a straight posture. In addition, the acceleration of the dauer larvae after leaping was observed. To calculate the leaping speed and acceleration of this dauer larvae, image analysis with Fiji and Numpy was performed. The time evolution of the dauer larval position and leaping speed obtained by image analysis is shown in Figure 5.7. The time series data indicate

that the takeoff speed, defined as the leaping speed 0.05 ms after the dauer larvae completely leave the substrate, is typically 0.86 ± 0.07 m/s at E = 457 kV/m and that the takeoff speed increases with increasing E. Interestingly, dauer larvae have a finite speed before take-off (Figure 5.8, labeled as t = -0.05 ms), which I hereafter denote v_b. The nonzero value of v_b means that before the leap, the nematode moved slightly upward, i.e., the contact area of the tail with the substrate gradually decreases before the take-off. The value of the acceleration a in the air was also obtained by the time series data of speed at each electric field intensity (Figure 5.9). I observed that the increase in E increased the value of a. Note that the acceleration is not linearly proportional to E, which suggests that the charge Q charged on the leaping nematode was not constant concerning E but changed with E.

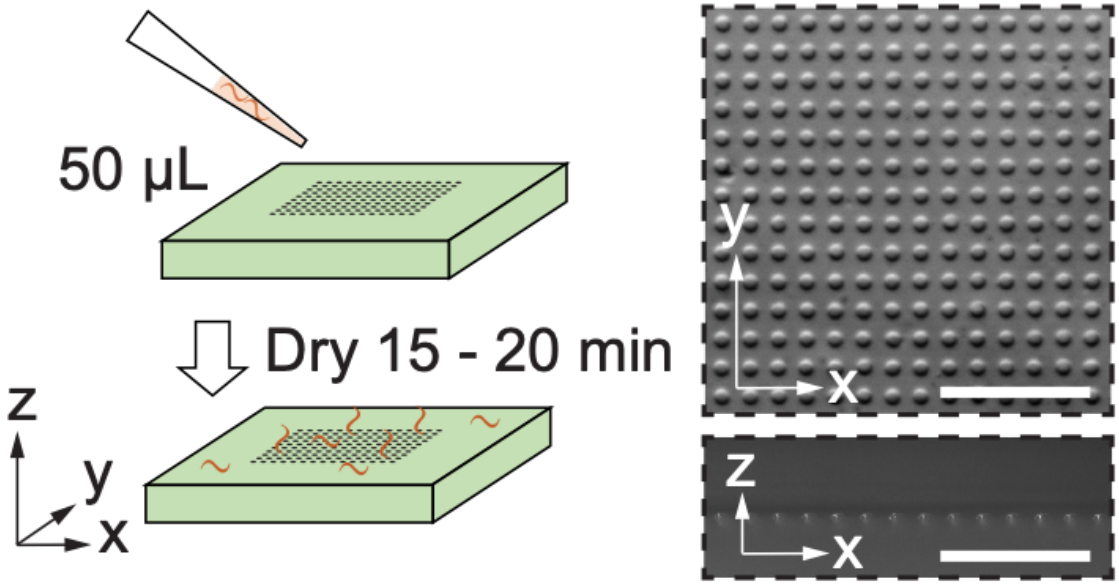

Figure 5.1 Experimental setup and the structure of MDS. Scale bar, 0.5 mm.

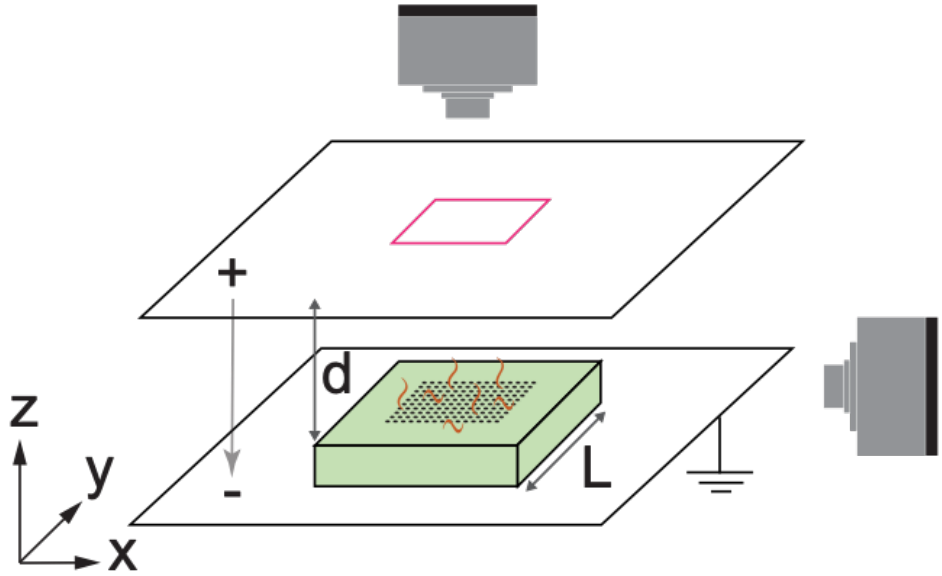

Figure 5.2 Leaping observations of *C. elegans* dauer larvae under an applied voltage. Images of dauer larvae leaping in response to an electrostatic field were obtained from above (areas of magenta squares). The leap-like transfer of individual dauer larvae was captured from the horizontal direction.

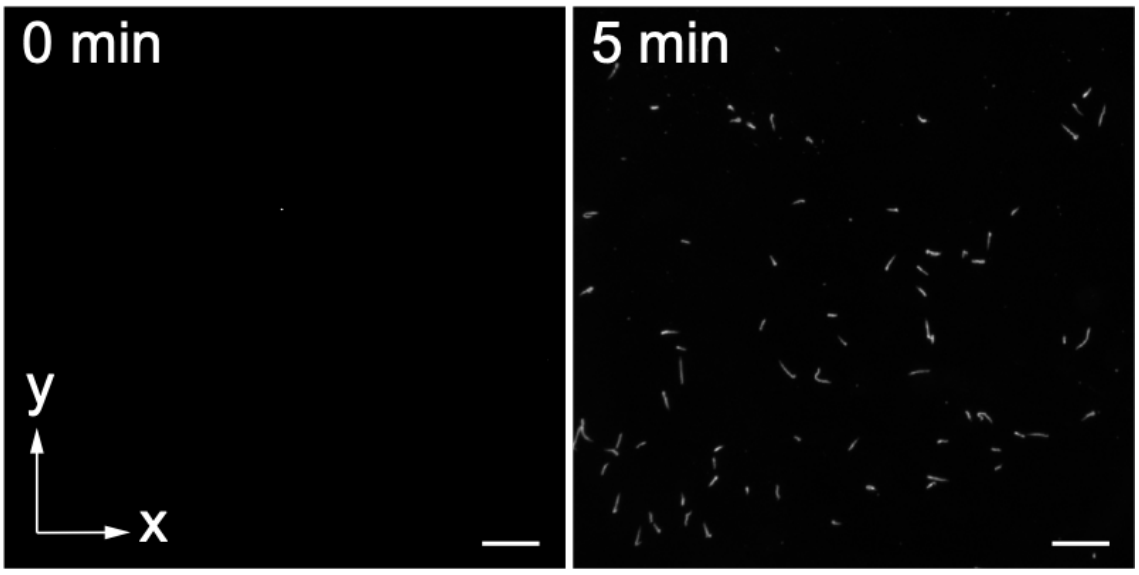

Figure 5.3 Images taken from above the ITO glass (the upper electrode). The left image is before applying an electric field, the right image is after 800 kVm^{-1}. Scale bar, 1.0 mm.

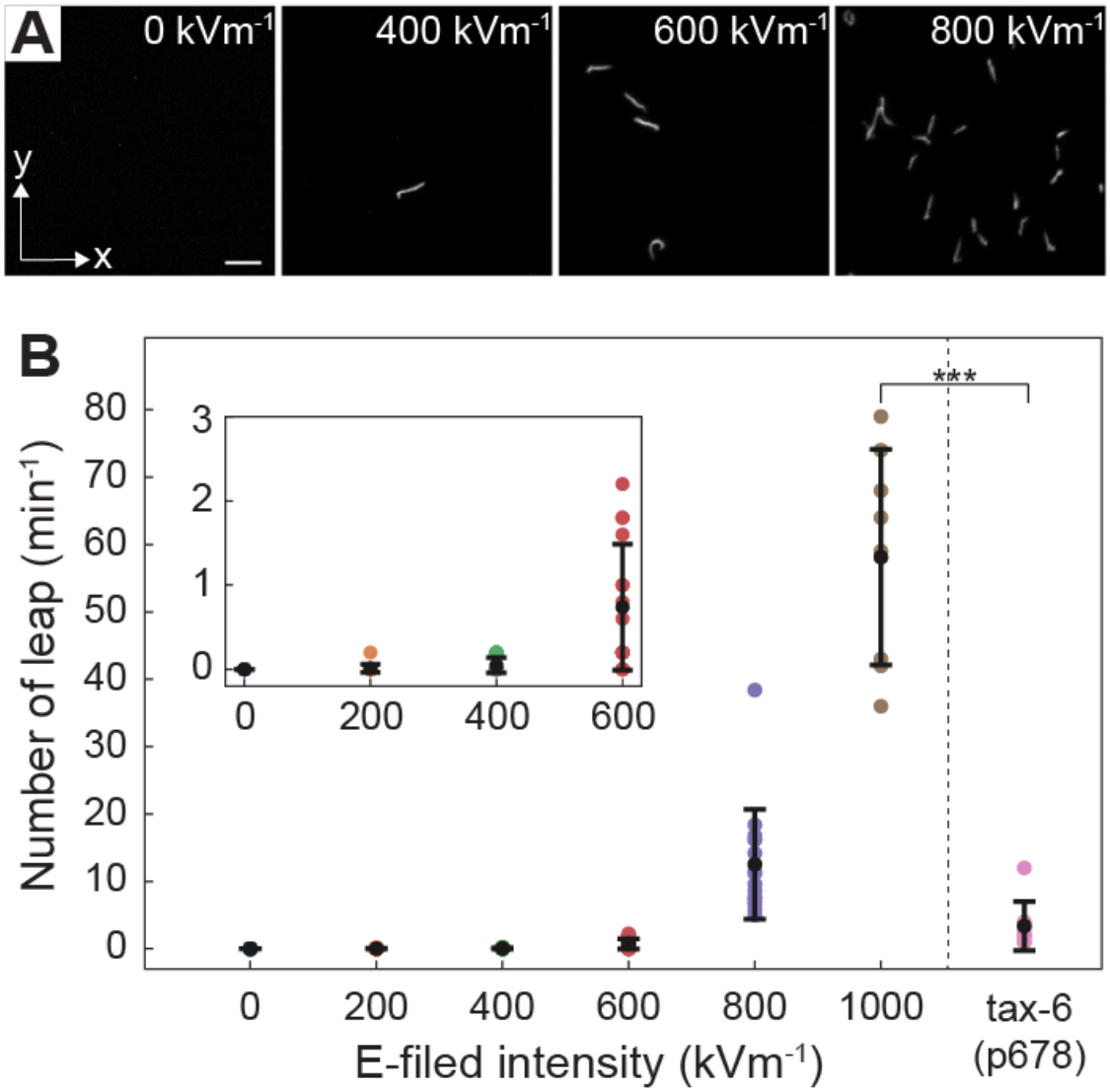

Figure 5.4 Relationship between electrostatic field intensity and the number of leaped dauer larvae. (A) The dauer larvae were seen from above the ITO glass (upper electrode) after electrostatic field application. Scale bar, 0.5 mm. (B) Leaping rate of dauer larvae at each electric field intensity. Black dots represent the mean; error bars represent the SEM. 16 experiments under 0, 200, 400, and 600 kVm^{-1} electrostatic field intensity conditions and 15 experiments under 800 kVm^{-1}. 8 experiments for wild-type *C. elegans* (N2) and *tax-6* (p678) mutants defective in electric field sensing under 1000 kVm^{-1}. *$p < 0.001$ based on unpaired t-test with Welch's correction. (A, B) In these experiments, the distance between the glass electrode and MDS was 4.49 ± 0.28 mm (mean ± SD, N = 95).

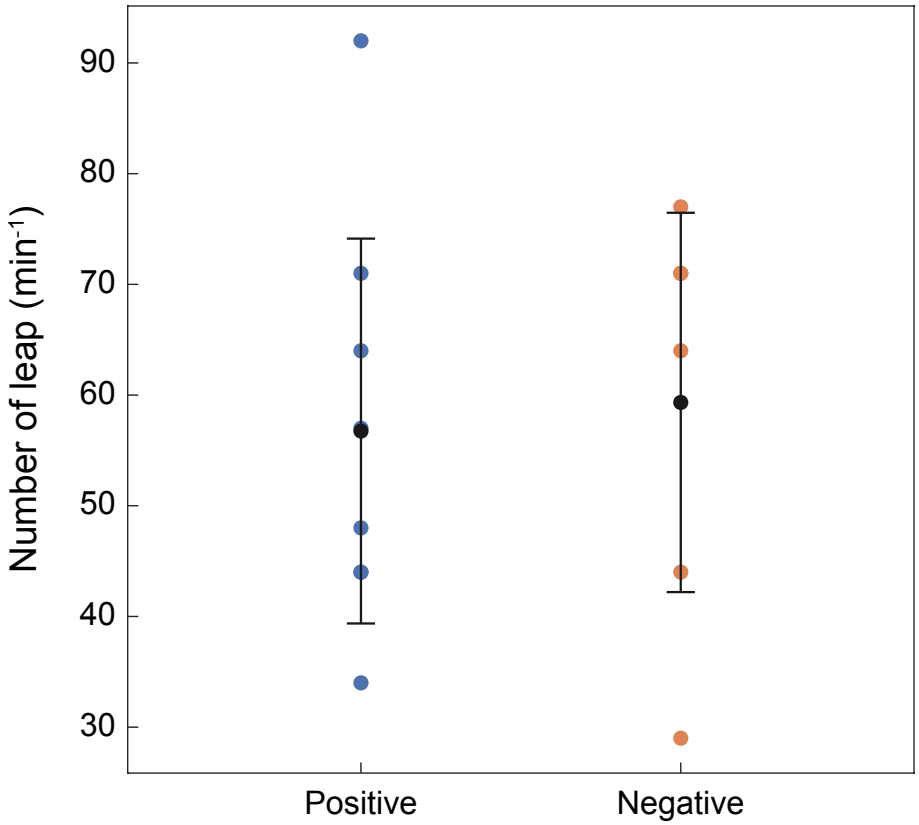

Figure 5.5 Relationship between the positive and negative directions of the electrostatic field and the leaping rate. Both positive and negative electrostatic field intensity is 800 kVm^{-1}. Black dots represent the mean; error bars represent the SD. 8 experiments for positive conditions and 6 experiments for negative conditions.

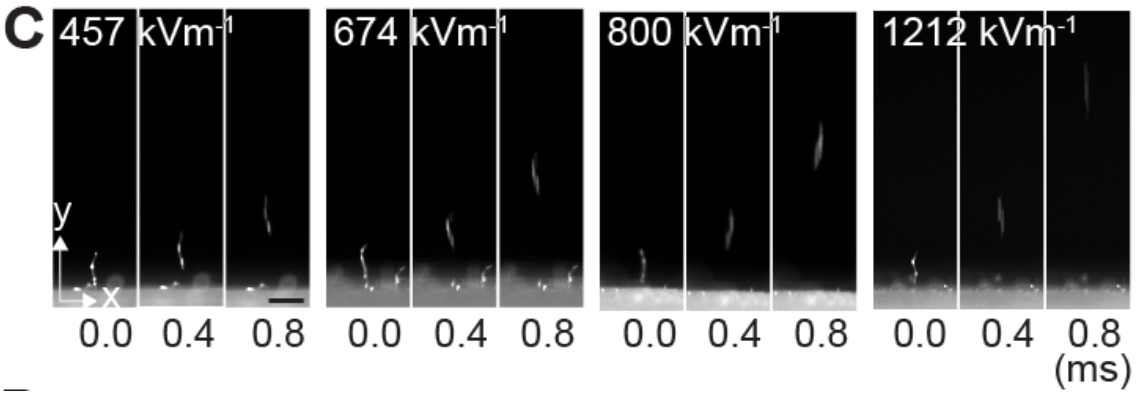

Figure 5.6 Dauer larvae leaping trajectory at each intensity. Scale bars, 0.5 mm. See also Video S7. In these experiments, the distance between the glass electrode and MDS was approximately 6.5 mm.

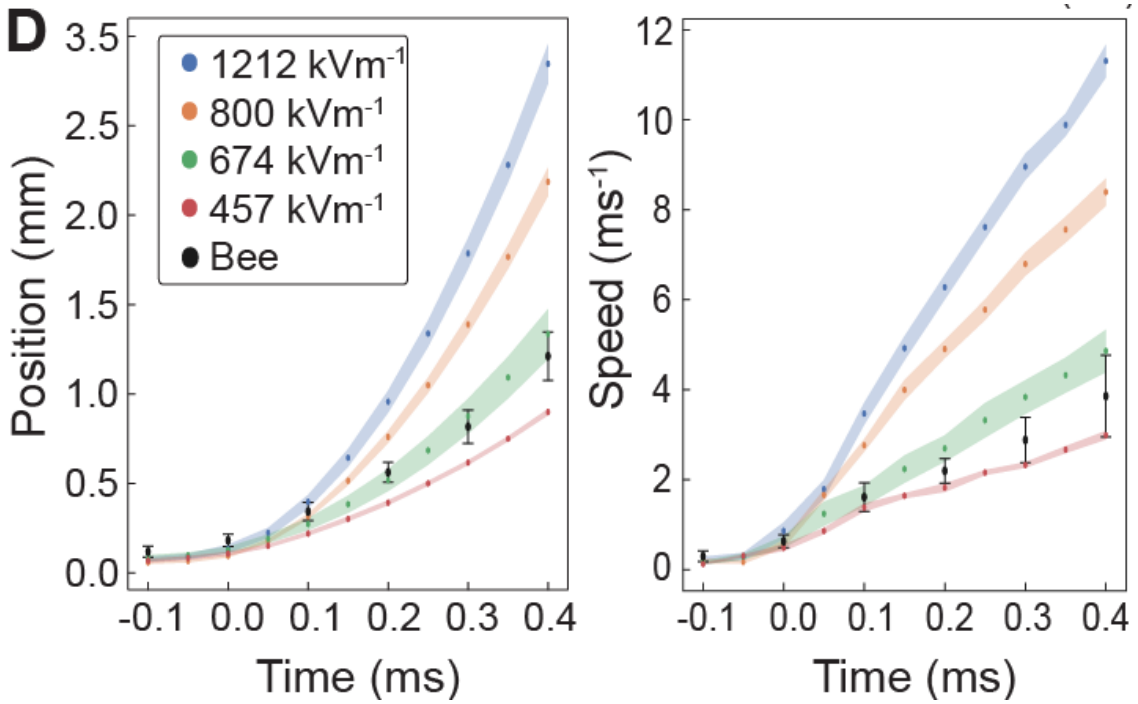

Figure 5.7 Time evolution of the leaping distance of dauer larvae (left) and the leaping speed of dauer larvae at each time point (right). Black dots are obtained from leaping for bumblebees. Dots represent the mean; error bars represent the SEM. In these experiments, the distance between the glass electrode and MDS was approximately 6.5 mm.

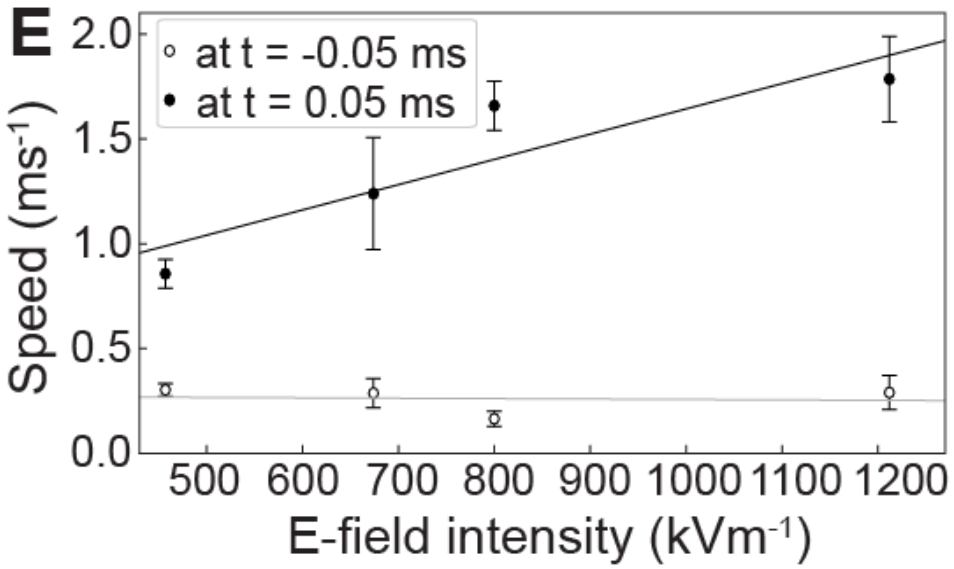

Figure 5.8 The speed of dauer larvae. The open dots are the speed before the leap v_b, and the closed dots are the take-off speed of the leap. I set t = 0 for the time the worm leaped. In these experiments, the distance between the glass electrode and MDS was approximately 6.5 mm.

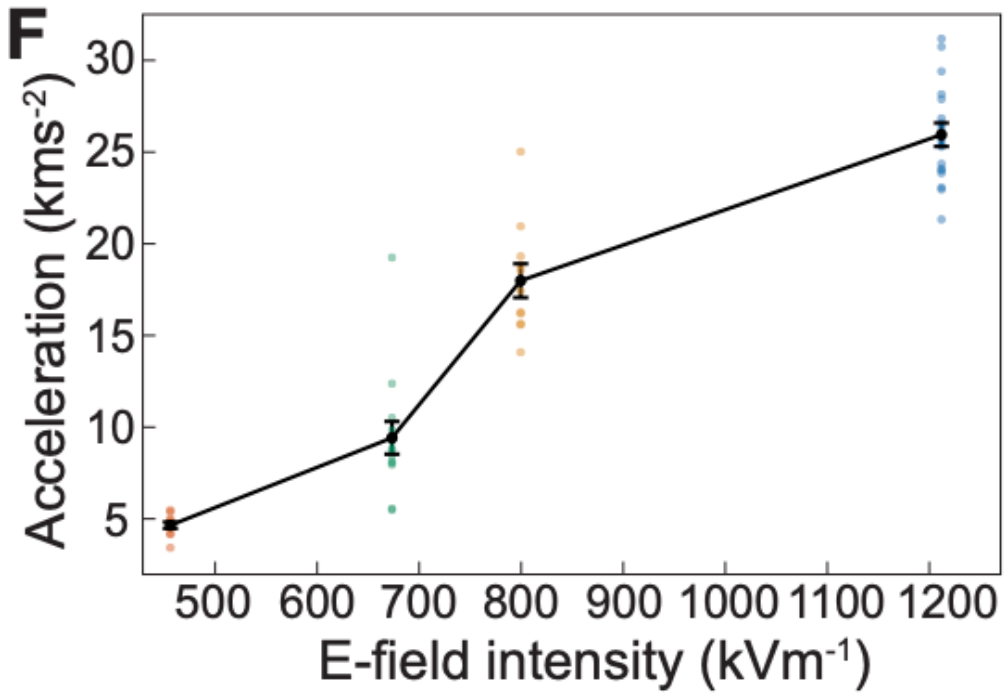

Figure 5.9 Relationship between electric field intensity and the acceleration of leaping (left). The acceleration of leap for bumblebees is plotted on the right. The distance between *C. elegans* daure larvae and bumblebees was 1.26 ± 0.46 mm (mean ± SD, N = 6). Black dots represent the mean; error bars represent the SEM. In these experiments, the distance between the glass electrode and MDS was approximately 6.5 mm.

5.3. Conclusion.

In this chapter, I quantitatively measured the relationship between fast transfer and electrostatic interaction by constructing an imaging system that can observe *C. elegnas* fast transfer under electric field control. The results showed that the leaping frequency of *C. elegans* increased with increasing electric field intensity (Figure 5.4). I also confirmed that *C. elegans* does not leap under conditions where no electric field is present (Figure 5.4). Interestingly, dauers leaped for both positively and negatively charged substrates (Figure 5.5). This indicates that the water film on the body surface can be charged due to dielectric separation. In addition, I observed that the higher the electric field intensity, the greater the acceleration of the leaping *C. elegans* (Figure 5.6). These experimental results indicate that the fast transfer of *C. elegans* is caused by electrostatic interactions.

Previous studies have shown that *C. elegans* can perceive electric field stimuli [25–27]. To investigate the effect of this electric field perception on fast transfer, I experimented with comparing the leap frequency between mutants defective in electric field sensitivity and wild strain N2. As a result, I found that tax-6 (p678) leaped less frequently than wild strain N2 (Figure 5.4). Since the Coulomb force was observed to be applied to *C. elegans* before leaping (Figure 5.8), it is possible that fast transfer is induced by the nictating nematode perceiving the electric field and performing some active behavior. In the next section, based on the results of the demonstration using insects, I discuss whether the fast transfer of *C. elegans* by electrostatic interactions can function as a phoretic behavior in nature.

6. Phoretic behavior of *C. elegans* daure larvae using electrostatic fields

In the previous chapter, I found that the leap-like transfer of *C. elegans* dauer larvae is induced by an electrostatic field. In addition, mutant experiments suggest that nictating nematodes may detect electric fields. Previous studies have suggested that nematode nictation is a behavior for attachment to a phoretic host. Our examinations further showed that only nictitating dauer larvae were capable of leaping. Therefore, this leap-like transfer of *C. elegans* dauer larvae might be expected to be a phoretic behavior similar to nictation. Through nictation (phoretic behavior), nematodes attach to host organisms and disperse into the environment. Known phoretic host organisms include insects, isopods, and snails [12 11]. Among these host organisms, insects have been found to be charged in nature [33]. The leap-like transfer of *C. elegans* dauer larvae induced by an electrostatic field may therefore be an effective phoretic behavior using the charge of the phoretic host. In this chapter, I demonstrate that *C. elegans* dauer larvae can attach to insects by using the leap-like transfer induced by the electrostatic field. I then explore the possibility that the leap I discovered is used in nature as a phoretic behavior.

6.1. Observation of leap-like transfer to the ground-crawling insect.

6.1.1. Methods.

First, I attempted to observe the leaping of *C. elegans* dauer larvae for the ground-crawling insect. The nematodes were *C. elegans* dauer larvae mass cultured by DFM as described above. As phoretic hosts, I used isopods (rough woodlouse), which have been confirmed to have a phoretic relationship with nematodes [11]. Dauer larvae mass cultured in DFA were collected from the lid of the petri dish with 1 mL of ultrapure water. Approximately 50 μL of this nematode suspension was dropped onto the MDS and excess water was removed. The MDS and the rough woodlouse were placed in a chamber (8 cm x 8 cm x 8 cm) made of acrylic plate, and the nictating dauer larvae were observed closing to isopods. An image of the dauer larvae phoretic behavior, viewed from the side, was obtained by a high-speed camera (FASTCAM Mini AX50, Photron) with a macro lens (3554B001, CANON) at 10,000 fps.

6.1.2. Results.

In this observation, it was not confirmed that *C. elegans* leaped on the isopods. On the other hand, I could also observe incidental failures of phoretic behavior by nictaion. As a result of the observation, images of a nictating *C. elegans* dauer larvae contacting a rough woodlouse were obtained (Figure 6.1, Video S8 and Video S9, n = 4). Figure 6.1A and Video S8 show contact to the antennae and Figure 6.1B and Video S9 shows contact to the legs. However, in all cases, dauer larvae were unable to adhere to rough woodlouse. Further contact with the abdomen was found to be difficult due to the distance involved in nictation by a single worm.

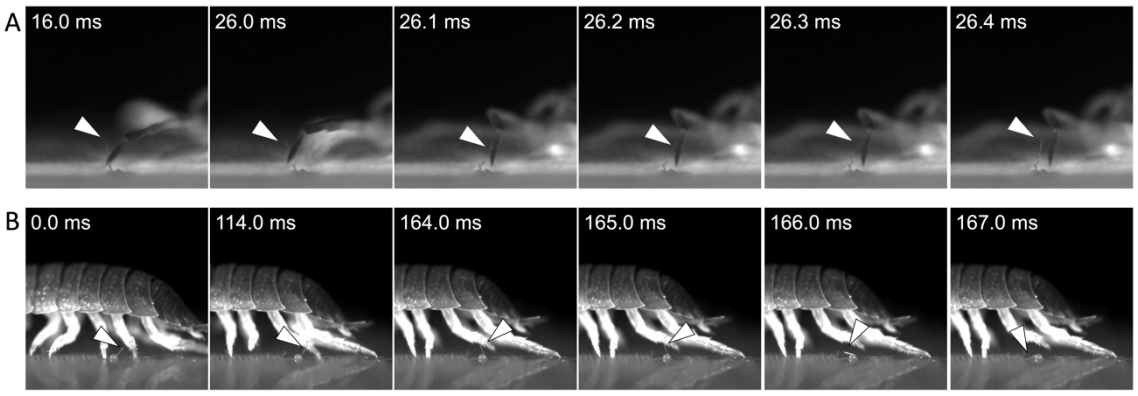

Figure 6.1 Nictating *C. elegans* contact with a rough woodlouse isopod. (A) Contact of *C. elegans* with the antennae. The nictating *C. elegans* was unable to adhere to the antennae and bounced at 26.4 ms. See also Video S8. (B) Contact of *C. elegans* with the legs. This nictating *C. elegans* also failed to adhere to the legs and was bounced at 167.0 ms. See also Video S9.

6.2. Observation of leap-like transfer to the aerial insect.

It was unable to observe *C. elegans* transfer to ground crawling insects by leaping. This is thought to be because ground-crawling insects are always in contact with the ground, which leaks the electric charge, resulting in a smaller charge. Compared to these terrestrial insects, aerial insects stay longer in the insulating air and thus have a greater chance of being charged than terrestrial insects. Most of the insects whose charge has been measured are aerial insects [24]. Therefore, I can expect to observe a fast transfer of *C. elegans* if the demonstration uses aerial insects. In this section, I summarize the results obtained on the observation of *C. elegans* fast transfer using aerial insects, the bumblebee.

6.2.1. Methods.

I examined whether *C. elegans* can leap and attach to charged aerial insects. As aerial insects, I used bumblebees (*Bombus terrestris*), because their charge in nature has been measured [34]. To observe leap-like transfer, I first prepared a DFA with a large number of dauer larvae nictating. The reason why MDS was not employed as the basis for nictation is that the frequency of nictation of dauer larvae is higher on DFA. The DFA base also facilitates the formation of nictation column of several dozen worms, making it easier to observe multi-worm transfers. To prepare DFA and nictating dauer larvae, dauer larvae were first cultured in large amounts in DFM. A piece of DFA was newly placed on the NGM medium, which was used for mass culture and allowed to stand at 20°C for at least 12 hours. With this additional culture, the dauer larvae move to the new DFA and begin to nictate. This new DFA was used to observe leap-like transfer to bumblebees. During imaging, DFA was placed on 4% agar to prevent it from drying out.

Bumblebees were charged in two ways: by a power supply device and by friction with a material. In the method using a power supply unit, a polystyrene petri dish with aluminum foil inside was connected to the positive side, and the negative side was grounded. Freshly killed bumblebees with carbon dioxide were placed in a petri dish connected to the positive and a voltage of 8 kV was applied. This charged bumblebee was fixed with ceramic tweezers and brought close to the nictating dauer larvae. The images were taken under a stereomicroscope (Olympus SZX16) with a CMOS camera (ORCA-spark, C11440-36U, Hamamatsu Photonics) at 20 fps.

In the method of electrification by friction with materials, polystyrene (Styrofoam) and Canada goldenrod (*Solidago canadensis*) were used as materials to rub against the bumblebees. Frictional charging is thought to be caused by the transfer of electrons, ions, or nanoparticles from one to the other when two different insulating materials are rubbed together [35–37]. Then, materials are ranked according to whether they tend to generate a positive or negative charge when they come into contact with each other, which is called the triboelectric series. Polystyrene is on the negative side of the triboelectric series [38], while bumblebees are on the positive side [39]. Therefore, according to the triboelectric series, I can expect the bumblebee to be positively charged (and the polystyrene

negatively charged) when the bumblebee is rubbed with polystyrene. I also know that bumblebees get their charge by rubbing against flowers and other objects when foraging. To demonstrate more natural conditions, bumblebees were rubbed by a flower *S. canadensis*, which has been confirmed to have a foraging relationship with bumblebees (Figure 6.2). Figure 6.3 shows the sequence of imaging procedures. Carbon dioxide-anesthetized bumblebees were fixed with ceramic tweezers and rubbed with polystyrene (Styrofoam) or *S. canadensis*. Both wings and part of the long body hair were cut off to prevent the bumblebee from accidentally contacting the DFA and losing its charge. Adherent *S. canadensis* pollen was removed by blowing with a dust blower (ELECOM). Rubbed bumblebees were brought into proximity to the DFA and the leap-like transfer of dauer larvae was captured. A high-speed camera (FASTCAM Mini AX50, Photron) with a macro lens (3554B001, CANON) was used to capture images at 10000 fps. The position of the dauer larva extracted by the image intensity exhibited the velocity and acceleration of the leap using NumPy (ver. 1.17.2)[32].

Figure 6.2 Bumblebee collecting nectar on a flower (*S. canadensis*).

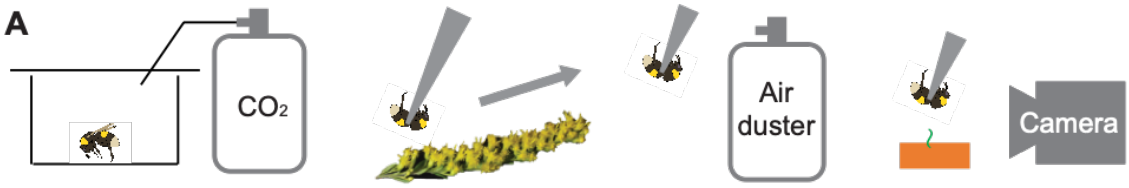

Figure 6.3 Procedures of this experiment. A bumblebee was charged by rubbing it against a Canada goldenrod *S. canadensis* and was then placed near a nictating dauer.

6.2.2. Results.

Leap-like transfer to bees charged with a power supply.

Figure 6.4 and Video S10, Video S11 show *C. elegans* dauer larvae leaping to a bee charged by a power supply. These leaping behaviors were observed in both the abdomen (Figure 6.4A, Video S10) and legs (Figure 6.4B, C, Video S11) of the bees. Interestingly, the *C. elegans* dauer larva, which leaped to the legs, was immediately nictated on the legs and leaped (Figure 6.4C). This observation suggests that dauer larvae are capable of nictation and leap-like transfer on insects that are phoretic hosts.

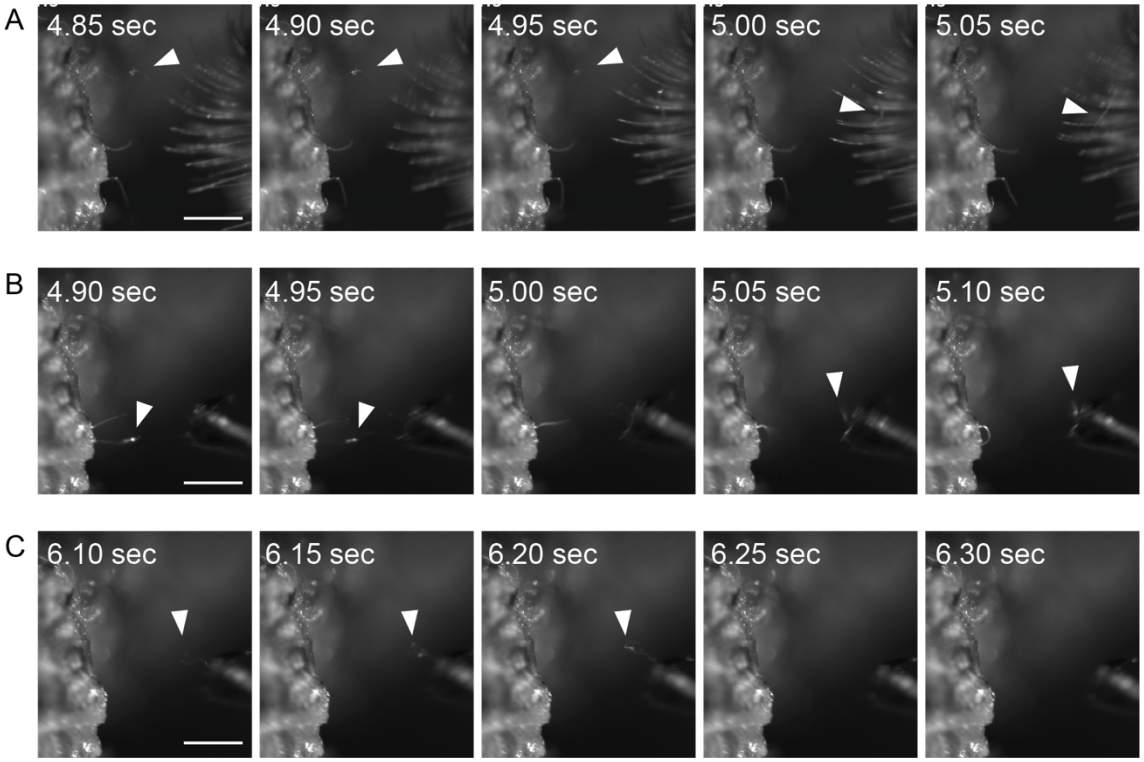

Figure 6.4 Leap-like transfer to bees charged with a power supply. (A) Leap-like transfer to the abdomen of the bee. Scale bar; 0.5 mm. See also Video S10. (B) Leap-like transfer to the leg of the bee. Scale bar; 0.5 mm. See also Video S11. (C) *C. elegans* dauer larva nictating on bee legs. The landing for the DFA could not be imaged, but this dauer larva leaped after nictation on bee legs. This dauer larva is the same individual that leaped to the legs in Figure 6.4B. See also Video S11.

6.3. *C. elegans* dauer larvae leap-like transfer to bumblebees charged by friction.

6.3.1. Results

I confirmed that dauer larvae can leap onto artificially charged bees, so I next tested whether the leap-like transfer can be induced by frictional charging, which is more similar to the natural state of the bees. First, I experimented with styrene foam (polystyrene), which is expected to give a negative charge to bumblebees based on the triboelectric series. From the results, it was confirmed that the dauer larvae leaped when the rubbed bees were approximately 1.0 mm closer to the nictating dauer larvae (Figure 6.5 and Video S12, Video S13, N=2). In both the power supply charge addition and styrofoam friction experiments, the leaping distance of dauer larvae was approximately 1 mm.

Last, I confirmed whether naturally charged bumblebees induce fast transfer of the dauer larvae. Since *S. canadensi*s is a flower that has a foraging relationship with bumblebees (Figure 6.2), I can expect bumblebees to have frequent contact with this flower in nature. Bumblebees rubbed with *S. canadensis* were brought approximately 1.0 mm closer to the nictating dauer larvae to observe the leaping of dauer. As a result, images of dauer larvae leaping on bumblebees were captured (Figure 6.6 and Video S14, N=6). From the trajectories of the leaping, I calculated the acceleration value of the nematode in the air. The mean value of acceleration was 12.8 ± 3.5 km/s^2 (Figure 6.7). The maximum leaping distance of this *C. elegnas* dauer larvae to the bumblebee were approximately 2.4 mm (Figure 6.8, Video S15). In addition, the worms attached to the bumblebee (Figure 6.9) survived for approximately 30 minutes on the bumblebee after the transfer. Interestingly, no leap-like transfer of dauer larvae was observed in this experiment when there was little or no *S. canadensis* pollen on the bumblebees after friction. This leaping of the nematodes occurred even when nematodes formed a nictation column (Figure 6.10, Video S16), where many dauers were stacked together to form a column and only one nematode was lifting the column of 80 dauers existed.

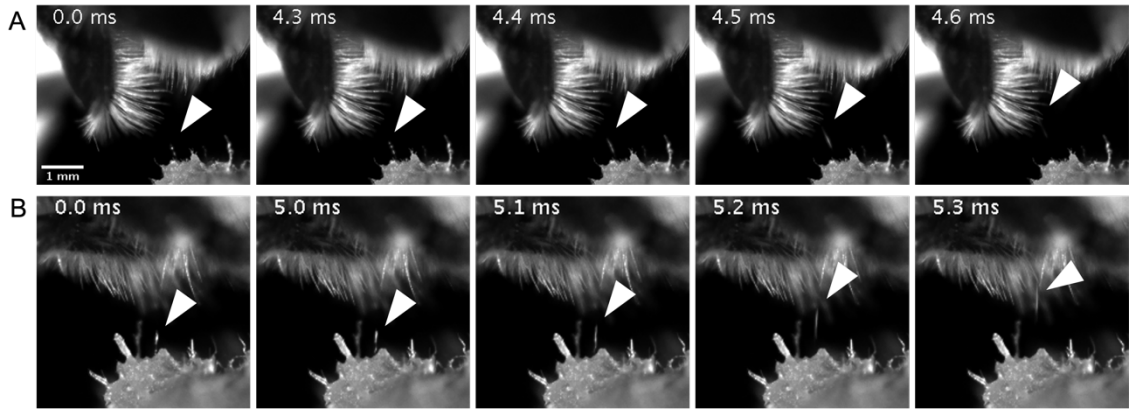

Figure 6.5 Leap-like transfer to bees charged with Styrofoam. (A) Leap-like transfer to the abdomen of the bee. Scale bar; 1.0 mm. See also Video S12. (B) Leap-like transfer to the body of the bee. Scale bar; 0.5 mm. See also Video S13.

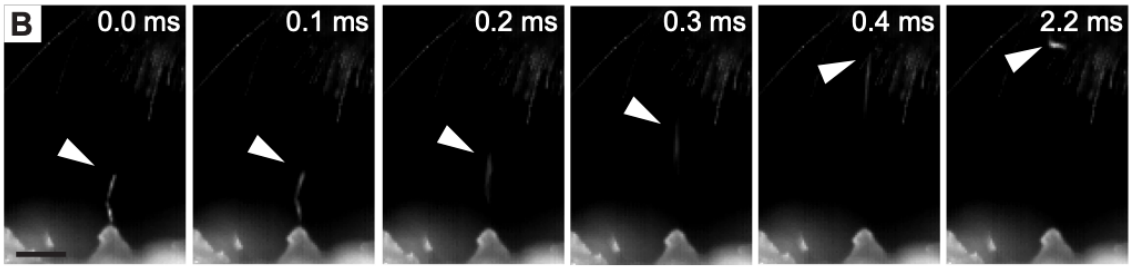

Figure 6.6 Leap-like transfer of *C. elegans* dauer larvae to bumblebees. A dauer nictating on DFA leaped to the charged bumblebee. Scale bar, 0.5 mm. In these images, the *C. elegans* dauer larva leaped approximately 1.3 mm. Scale bar; 0.5 mm. See also Video S14.

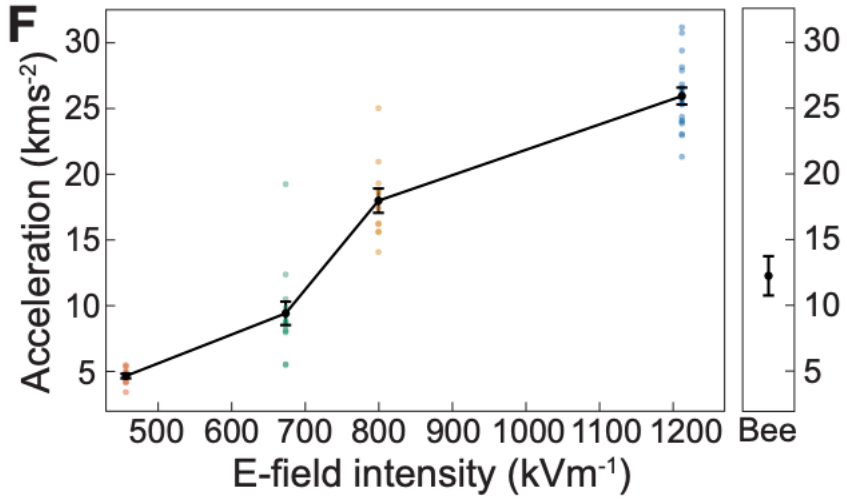

Figure 6.7 The acceleration of leap for bumblebees (right column). The left plot is the relationship between electric field intensity and the acceleration of leaping (same as Figure 5.9). From the comparison between the two plots, it can be inferred that the electric field intensity in this experiment was between 700 and 800 kVm^{-1}. The distance between *C. elegans* daure larvae and bumblebees was 1.26 ± 0.46 mm (mean ± SD, N = 6). Black dots represent the mean; error bars represent the SEM.

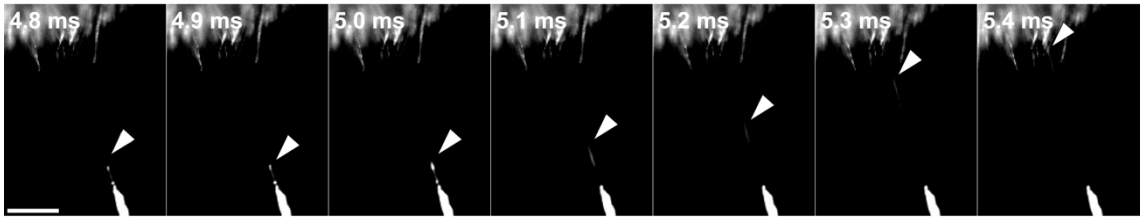

Figure 6.8 Long-distance leaping of *C. elegans* against bumblebees. A single dauer larva leaping from the tip of the nictation column of *C. elegans* dauer larvae onto a bumblebee. The *C. elegans* dauer larva leaped approximately 2.4 mm. This single-worm leap was taken just before the multi-worm leap in Figure 6.10 (Video S16). Scale bar; 1.0 mm. See also Video S15. The frame rate of recording is 10,000 fps.

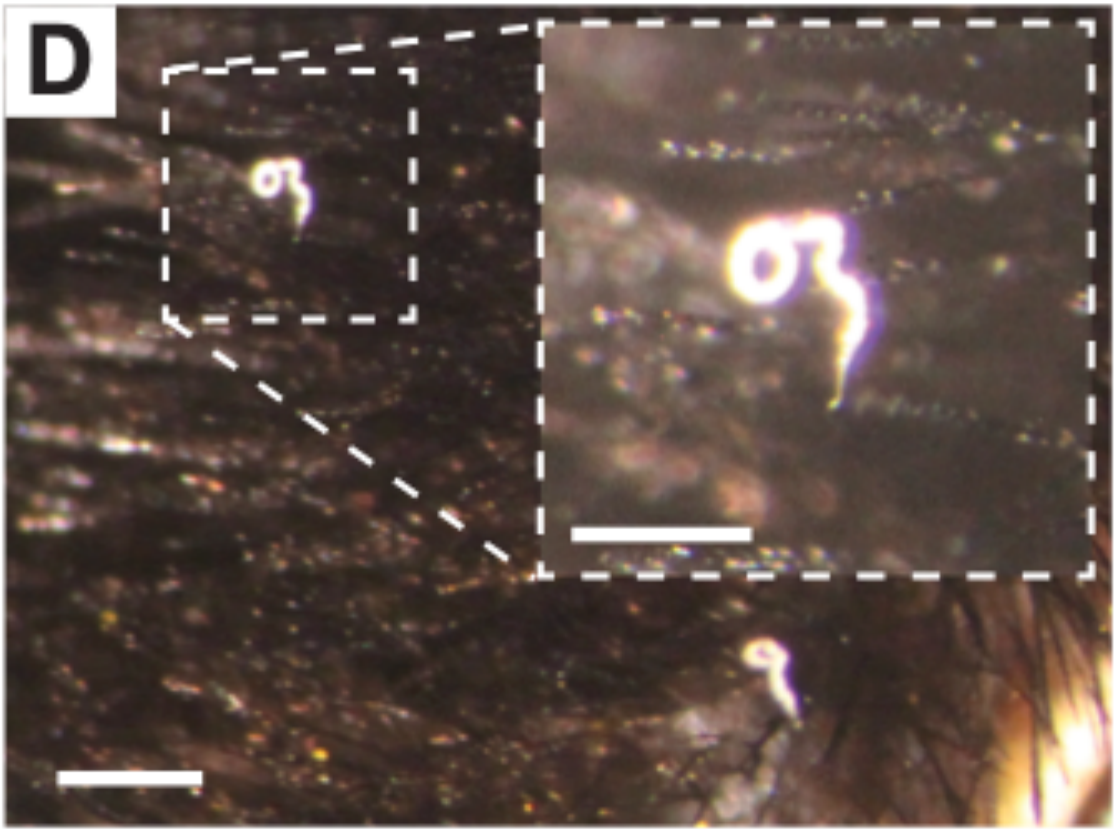

Figure 6.9 The attached *C. elegans* dauer larvae on the bumblebee. Scale bar, 1.0 mm.

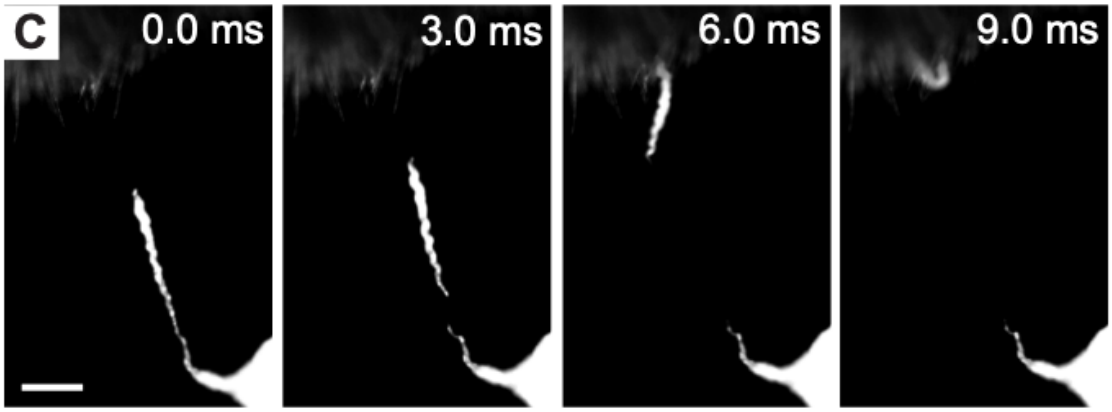

Figure 6.10 Leap-like transfer of a nictation column to bumblebees. A nictation column that consisted of 80 dauers leaped to the charged bumblebee from an edge of the DFA. In these images, the mass of *C. elegans* dauer larvae leaped approximately 2.2 mm. Scale bar, 1 mm. See also Video S16.

6.4. Calculation of bumblebee charge

From the observations in the previous section, I confirmed that *C. elegans* transferred for friction-charged bumblebees under more natural conditions. Next, I estimate the charge of the bumblebees used in the observations by using the finite element method (FEM). It is difficult to measure the amount of charge on a bumblebee at the moment the nematode leaps because the bumblebee's charge is gradually lost through contact with equipment and air. In addition, in the measurement of the amount of charge by a commonly used coulomb meter, the amount of charge on an object is lost by the measurement. For these reasons, I used FEM to estimate the amount of charge on the bumblebee when the nematode leaped, to measure the bumblebee's charge more accurately.

6.4.1. Methods.

To confirm that bumblebees frictionally charged with goldenrod were not overcharged, the amount of bumblebee charge was estimated numerically using the finite element method (FEM). The trajectories of the leaping nematode indicated that the acceleration value of the nematode in the air was a = 12.8 ± 3.5 km/s^2 (Figure 6.7). Comparing this value and those obtained in the MDS experiment (Figure 5.9), I estimated the value of E made by the charged bumblebee around the position where the nematode existed as E = 724 kV/m. From this value of E, I also estimated the charge on the bumblebee by numerical calculation using the finite element method (FEM). In detail, the bumblebee was approximated as a conductor sphere with a diameter of 12 mm and a distance from the ground (20 mm x 20 mm x 1 mm) of 1.26 mm. The initial values for all surfaces were set to V = 0. In a steady state, the ground potential was set to V=0 (grounded), and the bumblebee potential was set to 978.88 V so that the intensity of the electric field created by the bee on the ground surface was 724 kV/m. The boundary conditions for the other surfaces were set to $n \cdot D = 0$. Under these boundary conditions, the surface charge density (μC/m^2) of the bee was calculated by solving Maxwell's equations ($\nabla \cdot D = \rho$, $E = -\nabla V$) using the finite element method, and the charge (pC) of the bumblebee was estimated by area integration.

6.4.2. Results.

The estimated value Q of charge on the bumblebee was 770 pC. Since the charge of bumblebees flying in nature is 116 ± 159 pC and the maximum value of Q observed in nature is approximately 900 pC [34], it was concluded that the bumblebee charged by friction with Canada goldenrod in this experiment had the same order of magnitude of charge as the bumblebees in nature.

6.5. Effect of pollen grains on bumblebee charging

In the bumblebee charging experiments in this study, the leap-like transfer of *C. elegans* dauer larvae was observed only when *S. canadensis* pollen was attached to the bumblebee. Since leap-like transfer is more frequent for more charged objects, it can be inferred that friction with pollen particles is more significant for bumblebees' charging than contact with flowers. To confirm the effect of pollen particles on the bumblebee's charge, the bumblebee was rubbed with two substrates, *S. canadensis,* and natural pollen, and its charge was measured.

6.5.1. Methods

The wings of dead bumblebees were cut off at the root to prevent breaking the specimens during friction. The bumblebee was rolled on a piece of grounded aluminum foil for about 10 seconds to remove the charge held by the specimen. The bumblebee's charge was measured with a Faraday cage coulomb meter (KQ-1400, NK-1001A, Kasuga denki) and was used as the pre-friction charge. Bumblebees were carefully removed from the Faraday cage with ceramic tweezers, which were then rubbed with the substrate.

The experiment was conducted in two ways: with *S. canadensis* and with natural pollen. For friction of bumblebees by *S. canadensis*, the bumblebee was rubbed 10 times (approximately 7-10 cm per stroke) with a pollen-covered flower cluster (Figure 6.11A). Pollen adherent to bumblebees (Figure 6.11B) was lightly blown off with a dust blower, and then the amount of charge was measured by a Faraday cage. *S. canadensis* pollen was then removed with a dust blower and the same operation was performed to measure the amount of bumblebee charge due to friction with the flower cluster.

In the bumblebee friction with natural pollen, experiments were conducted using processed pollen products (KOHKAN Pharmaceutical Institute) made from dried pollen dumplings collected by honeybees. To prepare the substrate, 10 g of dried pollen dumplings were dissolved in 10 mL of pure water to form a paste. This was applied to a glass slide and allowed to dry at room temperature to fix the pollen to the glass. Bumblebees fixed with ceramic tweezers were rubbed 10 times (approximately 7-10 cm per stroke) with a prepared pollen slide, and their charge was measured in a Faraday cage. As a control experiment, the charge of bumblebees rubbed with glass slides was also measured in the same way.

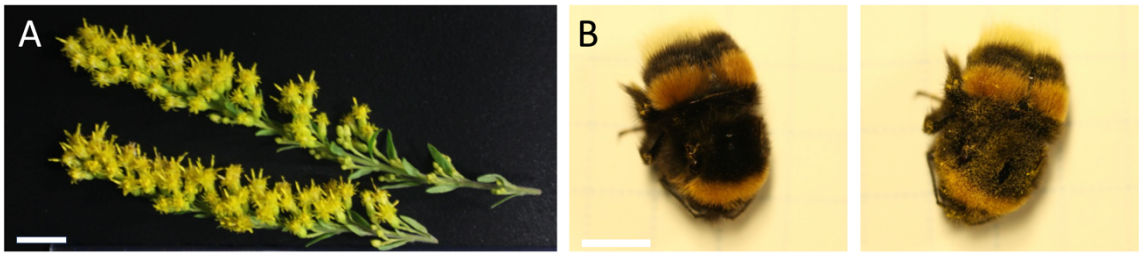

Figure 6.11 Photo of *S. canadensis* and *B. terrestris.* (A) Photo of Canada goldenrod (*S. canadensis*). Scale bar, 1.0 cm. (B) Photo of bumblebee (*B. terrestris*). The left image is before robbing and the right image is after robbing (covered by pollen particles). Scale bar, 0.5 cm.

6.5.2. Results

In both *S. canadensis* and natural pollen experiments, pollen grains were found to facilitate the charging of bumblebees (Figure 6.12). The charge of bumblebees rubbed with Canada goldenrod (*S. canadensis*) is 806 ± 350 pC (mean ± SD, N = 10). On the other hand, bumblebees rubbed with pollen-removed *S. canadensis* were hardly charged (-10.0 ± 10.5 pC, mean ± SD, N = 10). Experiments using natural pollen produced a greater charge than those using flowers. The charge of bumblebees rubbed with pollen (Glass +) was 2020 ± 340 pC (N=10), while the charge of bumblebees rubbed with glass substrate (Glass -) was 640 ± 110 pC (glass-, N=10).

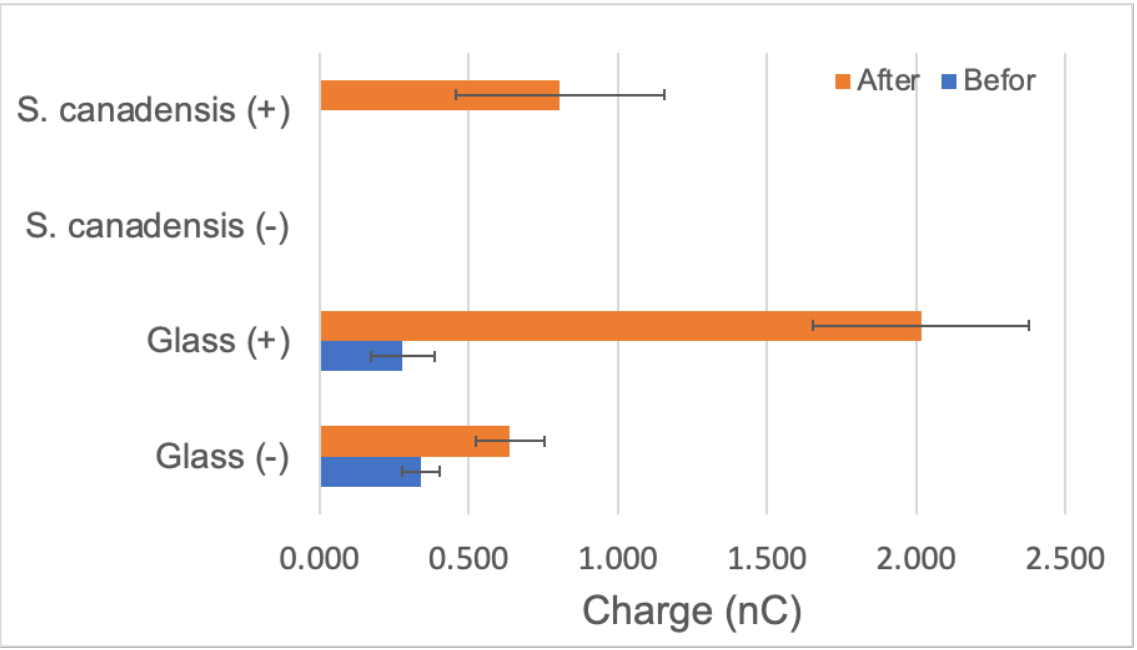

Figure 6.12 Charge of bumblebees due to friction with a substrate. The blue bars indicate the amount of charge before friction, and the orange bars indicate the amount of charge after friction. *S. canadensis* (+) and *S. canadensis* (-) refer to pollen-retaining and pollen-removed flower clusters, respectively. Glass (+) is the result of friction on a glass slide coated with natural pollen paste, and glass (-) is the result of friction by the glass slide itself.

6.6. Conclusion

In this chapter, I investigated whether fast transfer by electrostatic interaction can act as a phoretic behavior of *C. elegans* in demonstrations using terrestrial and ariel insects. In the demonstration using the terrestrial insect isopod, it was not possible to observe *C. elegans* adhering to the isopods by fast transfer. However, I was able to observe that nictating dauer larvae failed to attach to isopods. From Video S8, I can infer that the reason the dauer larvae could not ride the isopods was because they could not overcome surface tension with the ground. Thus, for *C. elegans* dauer larvae to adhere to host organisms by physical contact, the surface tension must be sufficiently low or the contact area with the host must be large enough. This suggests that nictating *C. elegans* does not always attach to a phoretic host by physical contact. The surface tension did not control in this examination because metabolites of *C. elegans*, components of the culture medium (NGM, DFA), and residual *E. coli* were not removed. Therefore, it is difficult to quantitatively evaluate whether this observational system exerts the same surface tension on the dauer larvae as in the natural environment. The dauer larvae, however, are often found in environments with abundant bacteria and metabolites from a range of organisms, such as decaying fruits [40]. From this demonstration, it appears that overcoming surface tension acting between ground and dauer larvae is an important factor in the phoretic behavior of *C. elegans.*

Next, I attempted to observe the fast transfer of *C. elegans* to ariel insects in a demonstration using bumblebees. As a result, I successfully observed *C. elegans* jumping on bumblebees rubbed with flowers. In this demonstration, *C. elegans* attached to bumblebees over a spatial gap of up to 2.4 mm. This distance is approximately five times the body length (0.5 mm) of *C. elegans* dauer larva. I also confirmed in a similar demonstration that a dauer tower consisting of approximately 80 dauer larvae can also jump on a charged bumblebee. These results indicated that *C. elegans* can jump on charged insects, then I next assessed whether the bumblebees used in these demonstrations were over-charged. First, I determined the electric field intensity formed by the bumblebee from the acceleration of fast transfer and then estimated the bumblebee's charge by FEM based on the value of acceleration. As a result, the bumblebee used in the demonstration was estimated to have a charge of approximately 770 pC. In addition, a method using a Faraday cage, which is commonly used to measure the amount of charge on an object, was also used to determine how much charge bumblebees get from friction with flowers. Measurements showed that the bumblebee's charge was 806 ± 350 pC immediately after friction with the flower. The higher values measured by the Faraday cage method than those estimated by FEM might be due to leaks of bumblebee charge by contact with equipment and air during observation. These estimated and measured bumblebee charge values are of the same order of magnitude as the natural bumblebee charge (116 ± 159 pC) and lower than the maximum (approximately 900 pC) [34]. Thus, it was confirmed that the bumblebees used in the demonstration did not have a charge large enough to be found in nature. Therefore, the fast transfer found in this study

could be used in nature as a phoretic behavior of *C. elegans* that utilizes the electric charge of the phoretic host.

7. Discussion and conclusion

In this study, I first noticed that the long-used model organism *C. elegans* transfer across a gap while nictating on a protruded substrate. Further examinations showed that electrostatic interactions between *C. elegans* and the substrate caused the leap-like transfer. A single nictating worm can leap even if it carries multiple worms as a 'dauer tower', which I referred to as 'multi-worm transfer'. Finite element calculations combined with experiments showed that their interaction is dependent on the electric field and that the bumblebee has an electrostatic charge, which is comparable with that conformed by our measurements and observed in the wild. I thus demonstrated that the electric field could facilitate different species' interaction and dispersal.

Charles Darwin noted that animal dispersal was a critical determinant of species evolution and range expansion. In his last publication, he reported hitchhiking by a freshwater bivalve and remarked on the importance of a dispersal phenomenon now known as phoresy[1]. Phoresy is a type of interaction in which one species, the phoront, utilizes another species, the dispersal host, for transportation to new habitats or resources. However, dispersal mechanisms in the submillimeter world remain unexplained because of the small sizes of the animals (submillimeter) and the lack of suitable subjects for investigation of small-animal ethology; how are such small animals able to disperse, sometimes globally? *C. elegans* is a representative submillimeter organism and is known to be a cosmopolitan species that exhibits a type of phoretic dispersal behavior. Therefore, our finding of the leaping phenomenon provides a good opportunity to address the question regarding the small-animal world.

Previous studies have suggested that direct contact between the phoront and dispersal host is a mechanism underlying phoretic dispersal. Therefore, *C. elegans* nictation likely increases the frequency of contact with other animals and decreases the surface tension of the water acting on a worm and the ground, thereby enhancing the success rate of the phoretic attachment. This surface tension also affects the leaping of *C. elegans*, and I have confirmed that decreasing the surface tension that acts on *C. elegans* increases its leaping speed (Figure 3.5). Therefore, I can consider that the leap occurs when the Coulomb force is sufficiently larger than the surface tension. From the data in Figure 5.9, I can estimate a characteristic diameter d of the worm's tail contacting the substrate, at which the worm starts to move up because of the electrostatic force. By considering the force balance on the tail, I have the relation $\gamma d = F_e$, where γ is the surface tension between water and air and F_e is the electrostatic force acting on the worm. If I take the values $\gamma = 72$ mN/m and $F_e = ma = 5000*10^{-10}$ N, which is evaluated from the data at E=450 kV/m by assuming the mass of the worm is $m = 10^{-10}$ kg, I have $d = 6.9$ μm. Since the tail of the dauer larva of *C. elegans* is very thin toward its tip [41], it is expected that there is a case where the dauer reaches the value of d when nictating on a substrate.

Nematodes that exhibit nictation and jumping exist besides *C. elegans*, among which the insect parasitic nematode *S. carpocapsae* has been well-studied for jumping. Previous studies have estimated that *S. carpocapsae* infective juveniles leap by their power, and their acceleration during the take-off is approximately 1600 ms^{-2} [42]. This acceleration value is on the same order of magnitude as the leaping acceleration of *C. elegans* (approximately 4600 ms^{-2}, Figure 5.9), suggesting that the force required to overcome the surface tension is similar in both nematodes. Additionally, the force required for *S. carpocapsae* to leap into the air is calculated to be approximately 0.1-1.0 μN. This value is consistent with the force that can be produced by the muscles of *C. elegans* [43]. In addition, our research shows new roles for nictation in addition to its function of reducing surface tension. First, as the distance to the dispersal host decreases with the length of the nematode and the Coulomb force is inversely proportional to the square of the distance, nictation has the advantage of increasing the Coulomb force received from the dispersal host. In addition, since strong electric fields tend to form at projecting parts such as the corners of the agar and the head of the worm, this has the effect of strengthening the electrostatic interaction. Thus, I have discovered a new function of nictation, which enhances the effect of electrostatic interactions and thereby dispersal efficiency.

Electric fields are common in terrestrial environments[24] and used by organisms such as bees and spiders[44–46]. In addition, since bacterial cells and spores are negatively charged[47–49], the electric field in the environment may contribute to the dispersion of these microscopic organisms[24]. *C. elegans* has been found in nature to attach the ground-crawling organisms such as snails and isopods[11]. In our experiments, *C. elegans* dauer larvae leap toward both the positively and negatively charged substrates (Figure 5.5), indicating that they can carry a charge because of the induced charge of the water film on the body surface. On the other hand, snails do not likely to accumulate significant static electricity because they seem to have highly conductive body surfaces and usually inhabit moist or very humid environments. Therefore, I assume that physical contact between *C. elegans* and ground-crawling organisms is likely their major phoretic mechanism rather than electric interaction. Although *C. elegans* has not been reported to attach to bees, *C. elegans* is known to attach to flying insects such as moth flies in the wild [12]. Previous experiments also showed that *C. elegans* can disperse through attachment with house fly *Drosophila melanogaster* [5]. In addition, *C. japonica*, a related species of *C. elegans*, has been found to form a species-specific phoretic association with the shield bug (*Parastrachia japonensis*) [50,51]. These electrically insulated organisms tend to accumulate charge during flight [45,52–55], walking [56–58], and physical contact[59,60], where the typical value of charge on organisms was of the order of 100 pC [24]. When an object having a charge of 100 pC approaches the nictating worm to 1.5 mm, the electric field intensity generated by the charged object at the location of the worm is 399 kV/m, which exceeds the minimum electric field intensity, 200 kV/m (Figure 5.4), beyond which the worm leaps. Therefore, it is plausible that the organisms known to be attached by *C. elegans* are also charged in the wild. Furthermore, *C. elegans* is also known to be able to perceive

electric field stimuli [25–27], and I found that mutants with defects in electric field sensing exhibit a lower leaping frequency than wild-type *C. elegans* (Figure 5.4). Therefore, this electric field sensing capability might be used for phoretic attachment. Together with these previous and our findings, I assume that *C. elegans* uses electric interactions to attach insects including bumblebees in the wild. Because *C. elegans* is a model organism, unlike other submillimeter animals, genetic methods have been well established to study the relationship between behavior, neural activity, and genes. Therefore, further studies on the electric field and the behavior of *C. elegans* are expected to provide more details on the electrical ethology of microorganisms.

8. Acknowledgment

This doctoral thesis is a compilation of my research conducted during my enrollment in the doctoral program at the Department of Soft Matter, Graduate School of Life Sciences, Hokkaido University. I wish to express my heartfelt gratitude to Professor T. Nakagaki for his guidance as my advisor and for providing me with the opportunity to pursue this research. I am deeply thankful for his support. I also extend my appreciation to Professor H. Haga and Associate Professor K. Sato for their valuable advice and guidance on the finer details of this paper. Their input has been greatly appreciated. Additionally, I would like to thank Assistant Professor T. Nishigami for his assistance throughout various stages, including experimental design, implementation methods, and thesis writing. I am truly grateful for his support. Lastly, I would like to acknowledge the members of my laboratory for their assistance, from engaging in seminar discussions to providing consultations. I am thankful for their contributions.

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Supplemental videos legends

Video S1. Leap-like transfer of *C. elegans* daure larva in a Petri dish.

Video S2. Single-worm transfer in *C. elegans*. *C. elegans* that propagated in a dog food agar medium were collected using water and inoculated onto the dog food. In the video, a single worm nictates on the dog food and then jumps without any specific motion. The jump scene alone is repeated in slow motion in the latter half of the video. Figure 2.2A was generated from this video sequence. The frame rate of recording is 40,000 fps.

Video S3. Multi-worm transfer in *C. elegans*. *C. elegans* that propagated in a dog food agar medium were collected using water and inoculated onto the dog food. In the video, two worms exhibit column nictation on another worm. Then, one of the worms takes off using its tail and jumps together with the other worm. The jump scene alone is repeated in slow motion in the latter half of the video. Figure 2.2B was generated from this video sequence. The frame rate of recording is 40,000 fps.

Video S4. Multi-worm transfer in *C. elegans*. Multiple worms exhibit column nictation on the dog food. Then, one of the worms takes off using its tail and jumps together with the other worms. The jump scene alone is repeated in slow motion in the latter half of the video. Figure 2.2C was generated from this video sequence. The frame rate of recording is 20,000 fps.

Video S5. *C. elegans* accelerates after jumping. Figure 3.3 was generated from this video sequence. The frame rate of recording is 10,000 fps.

Video S6. Leaping dauer larva with curved trajectories. Nylon mesh was used as a scaffold for nematodes. Figure 3.6 was generated from this video sequence. The vertical axis is the z-axis. The frame rate of recording is 10,000 fps.

Video S7. Leap-like transfer of *C. elegans* at different electric field intensities. Figure 5.6 was generated from this video sequence. In these experiments, the distance between the glass plate electrode and the MDS was adjusted to approximately 6.5 mm. The frame rate of recording is 20,000 fps.

Video S8. Nictating *C. elegans* contact with the antennae of a rough woodlouse isopod. Figure 6.1A was generated from this video sequence. The frame rate of recording is 10,000 fps.

Video S9. Nictating *C. elegans* contact with the legs of a rough woodlouse isopod. Figure 6.1B was

generated from this video sequence. The frame rate of recording is 10,000 fps.

Video S10. Leap-like transfer to bees charged with a power supply. Figure 6.3A was generated from this video sequence. The frame rate of recording is 10,000 fps.

Video S11. Leap-like transfer to bees charged with a power supply. Figure 6.3B, and C were generated from this video sequence. The frame rate of recording is 20 fps.

Video S12. Leap-like transfer to the abdomen of bees charged with Styrofoam. Figure 6.5A was generated from this video sequence. The frame rate of recording is 10,000 fps.

Video S13. Leap-like transfer to the body of bees charged with Styrofoam. Figure 6.5B was generated from this video sequence. The frame rate of recording is 10,000 fps.

Video S14. Leap-like transfer of *C. elegans* on bumblebees. Figure 6.6 was generated from this video sequence. The frame rate of recording is 10,000 fps.

Video S15. Long-distance leaping of *C. elegans* against bumblebees. In this video, the mass of *C. elegans* dauer larvae leaped approximately 2.2 mm. Figure 6.8 was generated from this video sequence. The frame rate of recording is 10,000 fps.

Video S16. Leap-like transfer of a nictation column to bumblebees. Figure 6.10 was generated from this video sequence. The frame rate of recording is 10,000 fps.

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