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Short communication

Electrostatic measurement of dischargeable electricity and bioelectric potentials produced by muscular movements in flies

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ABSTRACT

A simple electrostatic apparatus was devised to measure dischargeable electricity and bioelectric potentials produced by flies. The apparatus involved two insulated electrodes, ICW(−) and ICW(+), oppositely charged with equal voltages supplied by two voltage-generators. In the electric field, the flies became net positive by instantaneously discharging their electricity and were attracted to negative surface charges on ICW(−). The tail-lifting movement by the attracted insect was an action creating electric potentials that could cause discharge of ICW(−). The discharge transiently appeared in response to individual movements and was larger when the tail was lifted at higher angles.

1. Introduction

The bioelectric characteristics of insects are a major focus in developing an electrostatic pest trapping technique for crop protection [1]. Of particular interest is to demonstrate their ability to generate an electric field to dispel the electrostatic attraction force of a trap. The cuticle layer covering the insect body is interesting because of its highly conductive nature [2–6]. Under the influence of an electric field, conductors are electrified as a result of uneven distribution of electricity (free electrons). This implies that insects experience an electrification of their surface cuticle layer in an electric field and that they would be forced to generate an electric potential inside their bodies to oppose the external electric potential of an electric field [7]. Our original idea was to devise a new method to evaluate the potential electric production by insects.

The main focus of this study is to compare insects to a biological voltage generator and to specify a power source for electric power generation. Some insects generate bioelectricity through muscular movement [4,5,8–10] and/or neural excitation [11]. Also in our previous work [12], we reported electric current-associated muscular movements by vinegar flies in an electric field; insect movements were clear enough to track the electric power generation linked to individual movements.

The electrostatic attraction force is safe for insects, and therefore is available for holding test insects on a probe of an electric current detector without causing any harm. Stable holding of an insect is essential to consecutively analyse a series of muscular movements and their corresponding current flows. We attempted to deprive test insects of the electricity in their cuticle layer in an electric field because electrification of the surface layer is thought to be harmless to the insect. This method is easier and safer than conventional microsurgical operations by which microelectrodes are inserted into muscular or nervous tissues [4,13]. Electrified insects became net positive and could be attracted to the cathodic pole used to form the electric field [1]. The necessary equipment for this experiment is simple. Basically, only three components (insulated wires for electrodes, voltage generators and current detectors) are needed.

Using this method, electrostatic measurements are implemented at pre- and post-attraction stages. The pre-attraction stage determines the amount of dischargeable electricity from an insect, when the insect electricity instantaneously discharged by the

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mechanically charged voltages (external voltages) is measured. The subsequent post-attraction stage determines the electric potentials generated by insect muscular movements, when the electrode-accumulated electricity mobilised by biologically generated voltages (internal voltages) is measured. Dynamic analysis of the video-recorded data of the attracted insects visualises electric current generation associated with individual muscular movements. We used three kinds of flies belonging to different families to comparatively analyse muscular movement-mediated electric power generation.

2. Materials and methods

2.1. Experimental equipment

Electrodes were constructed using two insulated conductor wires (ICWs; Fig. 1A). An iron wire (2 mm diameter, 2 cm length) was passed through a vinyl sleeve (1 mm thick; bulk resistivity, 1×10^9 Ω) to make an ICW. Two ICWs were paralleled at a 5-mm interval and linked to negative and positive direct current (DC) voltage generators (Max Electronics, Tokyo, Japan). The opposite ends of the ICWs were closed by inserting an insulator polypropylene rod (2 mm diameter, 5 mm length) into the sleeve. Both generators were linked to grounded lines, and a galvanometer PC7000 (Sanwa Electric Instrument, Tokyo, Japan) was integrated into each line. The generators were operated with 12 V storage batteries to supply equal negative and positive voltages to ICWs; negatively and positively charged ICWs are represented as ICW(−) and ICW(+), respectively. Cover sleeves were dielectrically polarised positively on the surface of the iron wire side and negatively on the outer surface of the insulator sleeve in ICW(−) and vice versa in ICW(+) (Fig. 1B) [14]. Opposite surface charges on the ICWs act as dipoles that form an electric field between them.

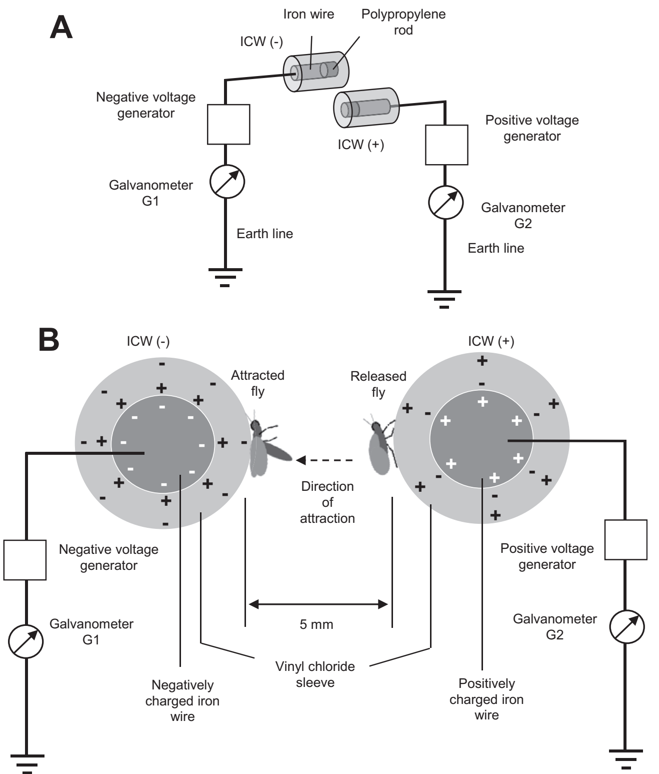

Fig. 1. Diagram (A) and cross section (B) of an electrostatic apparatus to measure dischargeable electricity and bioelectric potentials produced by muscular movements in flies. Two insulated iron conductor wires (ICWs) were oppositely charged with two DC voltage generators, and the direction and electric current magnitude were measured with galvanometers integrated into the grounded lines of the voltage generators.

2.2. Test flies

Three flies from different genera, humpbacked fly (*Megaselia spiracularis*, Schmitz: Phoridae), vinegar fly (*Drosophila melanogaster*, Meigen: Drosophilidae) and, greenhouse shore fly (*Scatella stagnalis*, Fallen: Ephydridae) were used as test insects. Pupae of test flies were purchased from Sumika Technoservice (Hyogo, Japan) and incubated for eclosion in a growth chamber (25.0 ± 0.5 °C, 12-h photoperiod of 4000 lux). The flies were reared following the method of Matsuda et al. [1], and newly emerged adults, 15–24 h after eclosion, were used as active flies for experiments. To collect insects, we constructed an insect aspirator consisting of a polypropylene tube (10 mm diameter) with a pointed tip (1 mm tip diameter). The opposite open end of the tube was linked to an aspirator (aspiration pressure 1.2 kg/cm^2). The insect was attracted to the pointed tip and released at a particular site on the ICW(+) (Fig. 1B) by stopping aspiration. All collected flies walked and flew normally and appeared to be unhurt by the collection. Body sizes of flies (length from head to wing edge) were measured using 30 adult test insects collected randomly: 4.02 ± 0.16, 3.68 ± 0.15 and 3.53 ± 0.26 mm for humpbacked fly, vinegar fly, and greenhouse shore fly, respectively.

2.3. Measurement of electric currents

2.3.1. Mechanical discharge

Both ICWs were oppositely charged with 1.0–9.0 kV to determine the range of voltages that cause mechanical discharge (constant transfer of electricity between both electrodes). In this electricity transfer, the direction and magnitude of electric current were measured with G1 and G2 galvanometers.

2.3.2. Insect discharge

Both ICWs were symmetrically charged, and adult flies were singly released at a particular site on ICW(+) to measure insect discharge (instantaneous transfer of electricity from an insect to ground) before the insect was attracted to the ICW(−). The current direction and magnitudes were detectable with a G2 galvanometer. Twenty adults were used per voltage and insect species.

2.3.3. Electric potential produced by insect movements

Both ICWs were charged with voltages causing no mechanical discharge. Electric currents linked to muscular movements by the attracted insect on ICW(−) were measured with G1 and G2. The profiles of the electric currents were recorded with a current detector (detection limit, 0.01 μA) integrated into the galvanometer (G2). The electric potential (voltage; muscular movement-derived electric power to mobilise electricity) was estimated based on a voltage–current calibration measure presented in this study. Movements by the attracted flies were video-recorded with a digital EOS camera (Canon, Tokyo, Japan) equipped with a dissecting microscope while applying voltages. Elevation angles of the tip lifted by the attracted insect were measured from video pictures, and the current magnitudes corresponding to individual tail-lifting movements were recorded. Movements were observed continuously (for 1 min) until the flies were removed from the ICW(−) after voltage to the ICW was stopped. All experiments were conducted at 25 ± 2 °C and 60 ± 3% relative humidity.

3. Results and discussion

3.1. Constant electric current by mechanical discharge

In this study, we formed an electric circuit in which the electricity moved from ground to ground (Fig. 1B). High voltages produced through a Cockcroft-circuit [15] of two voltage generators were used to electrify both electrodes by adding electricity to ICW(−) and pushing electricity out of ICW(+). The flow of the accumulated electricity in ICW(−) depends on the voltage applied to the electrodes, the electrode distance, the insulation resistance of the ICW cover sleeve and air conductivity between both electrodes. An electric current from an insulated conductor depends on the insulation resistance at a given voltage, which determines the level of insulator conductivity [16]. Air conductivity changes in response to changes in water-vapour concentration (relative humidity) in the air; the air conductivity becomes higher (i.e. higher amounts of electricity are transferred) under higher relative humidity [17]. The current was inversely proportional to increases in distance [1]. In this study, we set the distance between the ICWs to 5 mm because this distance kept the wings of the flies from ICW(−), even when they opened their wings on ICW(+). The voltage was changed to examine the voltage ranges that would cause a mechanical discharge. Eventually, a constant electric current occurred at >6.5 kV and became larger as applied voltage increased (Fig. 2).

3.2. Instantaneous electric current by insect discharge

The pre-attraction stage was to electrify an insect by passing the electricity out of the insect. Considering the highly conductive nature of the cuticle, the cuticle structure appeared to be a potential site for electrification in adults of the insect species used. Electricity transfer was detectable only by G2, indicating that the negative charge of the insects transferred to ground via ICW(+). Importantly, grounding the ICW(+) was essential to receiving a charge. In fact, cutting the grounded line resulted in loss of insect discharge and failure of the insect to be attracted to ICW(−) (data not shown). In all test flies, the amount of the discharged electricity was directly proportional to the applied voltage (Fig. 2A–C). At voltage ranges causing mechanical discharge, the current magnitudes were additive to those of the mechanical discharge. In all test flies, the regression analysis of plotted points provided two linear equations with different gradients. The equations at higher voltage ranges provided linear lines parallel to the line joining points of the current values of the mechanical discharge at given voltages. These results indicated that no more insect discharge occurred in these ranges, and therefore, that the maximum amount of dischargeable electricity could be estimated from the current values at the intercept of the two lines.

Table 1
Frequency of tail-lifting trials by test flies attracted to ICW(−) at different voltages and electric potentials produced by high-angle tail-lifting movements.

Test flies	Voltage (kV) applied to ICWs[a]	Total number of trials	Number of trials of high-angled tail-liting (80–90°)	Electric potentials (kV) produced by high-angled tail-lifting
Humpbacked fly	6.9	0.80 ± 0.41 a	0.30 ± 0.47 a	1.72 ± 0.16 a
	6.2	2.35 ± 0.49 b	1.30 ± 0.47 b	1.73 ± 0.13 a
	5.6	3.70 ± 0.47 c	2.45 ± 0.51 c	1.69 ± 0.12 a
Vinegar fly	6.9	1.55 ± 0.51 a	1.35 ± 0.49 a	1.54 ± 0.07 a
	6.2	3.40 ± 0.68 b	2.75 ± 0.44 b	1.52 ± 0.10 a
	5.6	6.35 ± 1.14 c	4.50 ± 0.76 c	1.49 ± 0.08 a
Greenhouse shore fly	6.9	1.00 ± 0.56 a	0.45 ± 0.51 a	1.39 ± 0.15 a
	6.2	2.35 ± 0.49 b	1.55 ± 0.51 b	1.41 ± 0.16 a
	5.6	4.05 ± 0.39 c	2.90 ± 0.55 c	1.34 ± 0.16 a

Frequencies and angles of tail-lifting by flies were determined from video pictures of 20 flies for each voltage and species. The different letters on the mean values in each vertical column of each species indicate significant difference ($p < 0.05$) according to Tukey's method.

[a] The highest, middle and lowest voltages stably attracting all test flies without causing mechanical discharge.

3.3. Transient electric current by insect muscular movements

The present voltage application (between 5.6 and 6.5 kV, causing no mechanical discharge) was sufficient to create an attraction force to ICW(−) for all test flies (Video supplement 1). The flies were released at a fixed site on ICW(+) and attracted to the same site on the opposite electrode ICW(−). The ICW attracted the flies in a supine position by capturing both wings.

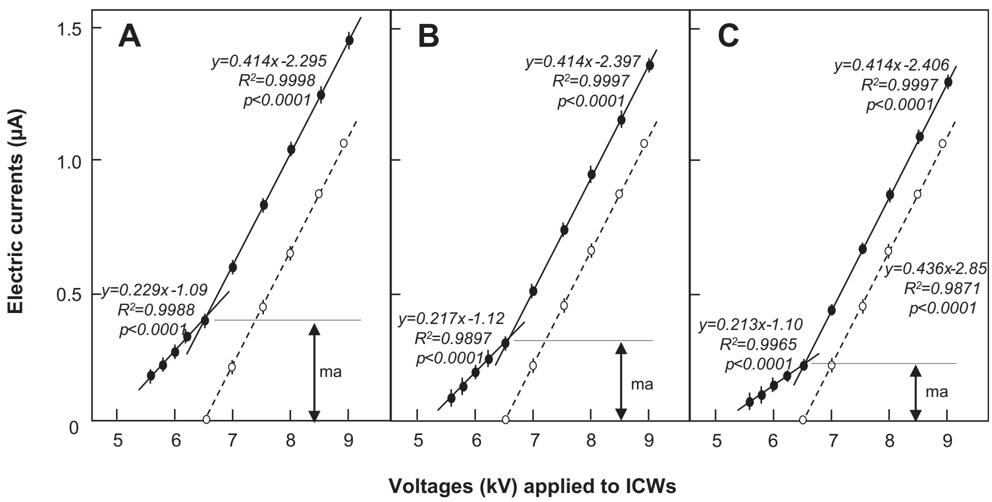

Fig. 2. Measurement of electricity discharged from adult humpbacked flies (A), vinegar flies (B) and greenhouse shore flies (C) in the electric field of the electrostatic apparatus. Twenty insects were used for each voltage, and mean values of the highest magnitudes of electric current instantaneously discharged from the insect were plotted with the S.D. Dotted line represents the mechanical discharge at given voltages. Two regression lines were provided from the plots, and the maximum amount (ma) of dischargeable electricity of the insect was given at the intercept of the two lines.

The attracted flies could not leave ICW(−) until the voltage was turned off. Possible movements by attracted insects were fluttering of the legs and lifting of tails (Video supplement 2). In this electric field, the flies were always exposed to the attraction force driven toward the ICW. The force was increased by application of higher voltages. Muscles were loaded with forces hindering their action, so the movements of flies were very slow and rare in response to higher voltages (Table 1). Under this condition, muscle fatigue was quick and the flies became motionless. From these results, the analysis of bioelectric potential generation by insect movement was conducted at the lowest voltage (at 5.6 kV) of this range with their most active movements. In the video display (Video supplement 2), we supplemented the electric current profiling data to show the close linkage of individual muscular movements with current generation. The current was detected when the flies lifted their tails, but not when fluttering their legs. Most importantly, the electricity transfer was simultaneously recorded with two galvanometers when the flies moved, and the magnitude of the current detected with the galvanometers was identical in all movements. These results indicated that the insects supply the voltages through movement to draw electricity from ICW(−) and conduct it to ground via ICW(+). At the same time, the lack of electricity in ICW(−) could be compensated by the electricity that the voltage generator drew from the ground. This was the most suitable interpretation to explain the simultaneous measurement of the same direction and magnitude of the electric current with the galvanometers integrated in the grounded lines. Electric potentials supplied by insect movements could be calculated using the voltage–current calibration measure shown in Fig. 3.

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.elstat.2013.10.012.

Electric potentials by tail-lifting were prominent enough to read the difference among different angles of the lifted tail (Video supplement 3). Angles were measured from the video pictures of 50 insects for each species, and then the corresponding current magnitudes were recorded. Fig. 4 shows a clear correlation between elevation angle and the electric potential

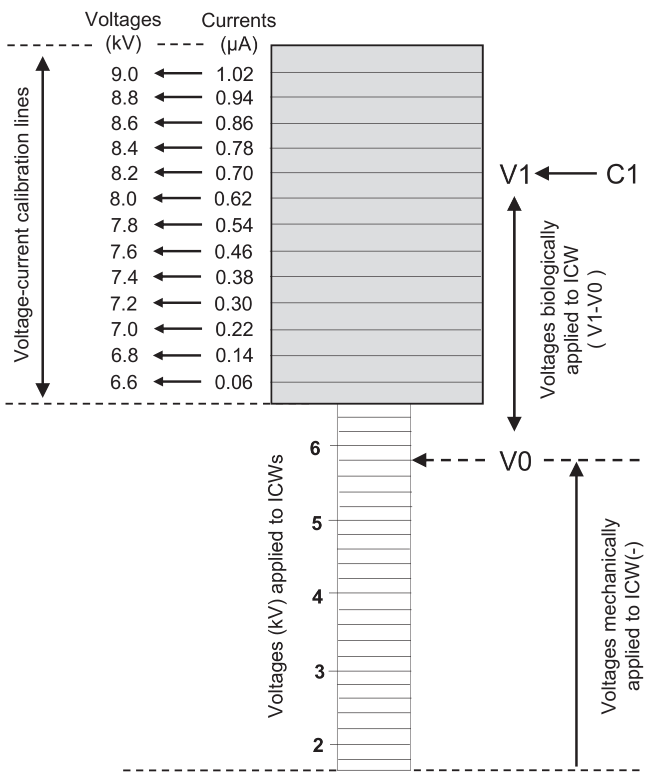

Fig. 3. A voltage–electric current calibration measure for calculating bioelectric potentials produced by muscular movements of test flies. The upper part of the measure shows the calibration lines based on the mechanical discharge in the electric circuit of the instrument (refer to Fig. 2) to determine the voltages that generate particular electric currents. First, the current magnitude (C1) produced by tail-lifting was measured and then the corresponding voltage value (V1) was read from the calibration line. The electric potential was calculated by deducting V0 (voltage of ICWs at the time of insect release) from V1: 8.2 − 5.8 = 2.4 kV in the case in the figure.

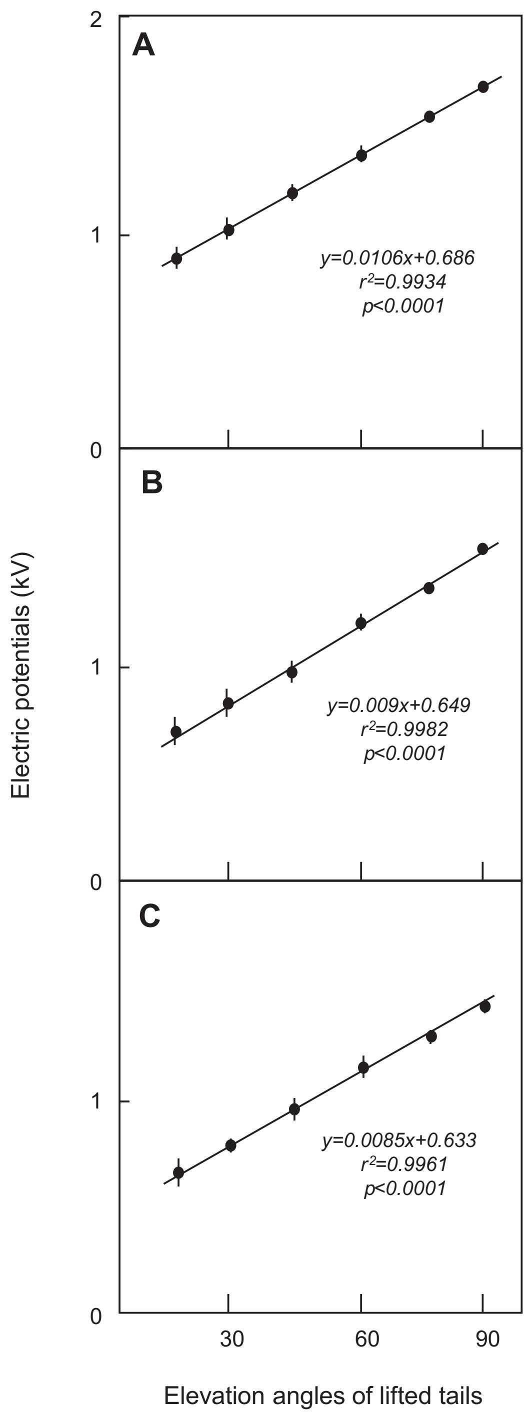

Fig. 4. Relationship between the elevation angles of tip-lifting movements and electric potentials produced by tail-lifting in humpbacked flies (A), vinegar flies (B) and greenhouse shore flies (C).

produced by tail-lifting in the three test flies. Thus, the present study indicates that lifting of the tail is a muscular action creating electric potential through bioelectric power generation. Table 1 also shows data for electric potentials produced by single tail-lifting movements with higher elevation angles (80–90°) at different voltages applied to ICWs. Obviously, all test flies produced the same level of electric potential by movement regardless of the applied voltage.

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In our study of insect movement, immobilised flies attempted to remove themselves from the ICW by dispelling the attraction force of ICW(−), i.e. by neutralising the positive charge in their body. The insect-derived voltage was sufficient to integrate the ICW(−)-accumulated electricity into the insect body. The maximum momentary bioelectric potentials of the test flies was 1.75 (humpbacked fly), 1.55 (vinegar fly) and 1.45 kV (greenhouse shore fly), generated by tail-lifting at the largest elevation angle (90°). However, the field strength of the electric field was strong enough that the electricity could be pushed out of the body without being used for neutralisation. Eventually, the insect was continuously held on ICW(−). Nevertheless, this situation did not harm the flies. In fact, tested flies walked and flew normally and could lay eggs (data not shown) after they were released from electrostatic holding.

4. Conclusion

We succeeded in measuring dischargeable electricity in flies and the bioelectric potentials produced by muscular movements using a simple electrostatic apparatus. In a particular range of voltages mechanically applied to ICWs, flies were electrified to the verge of discharging the electricity accumulated in ICW(−). This electrostatic situation discharged the electricity from ICW(−) once additional voltages were supplied by the flies attracted to ICW(−). In the electric field of the apparatus, the flies became net positive and were attracted to negative surface charges of ICW(−). The attraction was stable enough to consecutively trace muscular movements by the attracted insect. The tail-lifting movement by flies was a particularly prominent action creating electric potentials that could cause discharge of ICW(−). The discharge transiently appeared in response to individual movements and was larger at higher elevation angles of the lifted tip. The present method is safely applicable to a wide range of flies.

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