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Analysis of uncertainty of tracheal compression event classification

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Figure S1. Summary of temporal progression of collapse and reinflation events in the ventral tracheae of five specimens of *Platynus decentis*. The numbers refer to temporal order of events, progressing from 1 to 4. Collapse is shown in the top row, and reinflation in the bottom row, sorted in 5 columnar groups by individual. The left and right trunks are indicated. Each compression is color-coded to show occurrence of synchronous, down, up, out-in, in-out, and other patterns of temporal progression.
Table S1: Summary permutations of tracheal progression order by region.

| Direction    | N  | %    |
|--------------|----|------|
| Down         | 7  | 9.3% |
| In-Out       | 19 | 25.3%|
| No Direction | 22 | 29.3%|
| Out-In       | 19 | 25.3%|
| Synchronous  | 1  | 1.3% |
| Up           | 7  | 9.3% |

**Total** 75 100.0%
Table S2: Theoretical permutations of tracheal progression order by region.

| ID | Tube Region | Classification |
|----|-------------|----------------|
|    | I   | II  | III | IV  |               |
| 1  | 1   | 1   | 1   | 1   | Synchronous   |
| 2  | 1   | 1   | 1   | 2   | Up            |
| 3  | 1   | 1   | 2   | 1   | Out-In        |
| 4  | 1   | 1   | 2   | 2   | Up            |
| 5  | 1   | 1   | 2   | 3   | Up            |
| 6  | 1   | 1   | 3   | 2   | Out-In        |
| 7  | 1   | 2   | 1   | 1   | Out-In        |
| 8  | 1   | 2   | 1   | 2   | Other         |
| 9  | 1   | 2   | 1   | 3   | Other         |
| 10 | 1   | 2   | 2   | 1   | Out-In        |
| 11 | 1   | 2   | 2   | 2   | Up            |
| 12 | 1   | 2   | 2   | 3   | Up            |
| 13 | 1   | 2   | 3   | 1   | Out-In        |
| 14 | 1   | 2   | 3   | 2   | Out-In        |
| 15 | 1   | 2   | 3   | 3   | Up            |
| 16 | 1   | 2   | 3   | 4   | Up            |
| 17 | 1   | 2   | 4   | 3   | Out-In        |
| 18 | 1   | 3   | 1   | 2   | Other         |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
|19 | 1 | 3 | 2 | 1 | Out-In |
|20 | 1 | 3 | 2 | 2 | Out-In |
|21 | 1 | 3 | 2 | 3 | Other |
|22 | 1 | 3 | 2 | 4 | Other |
|23 | 1 | 3 | 3 | 2 | Out-In |
|24 | 1 | 3 | 4 | 2 | Out-In |
|25 | 1 | 4 | 2 | 3 | Other |
|26 | 1 | 4 | 3 | 2 | Out-In |
|27 | 2 | 1 | 1 | 1 | Down |
|28 | 2 | 1 | 1 | 2 | In-Out |
|29 | 2 | 1 | 1 | 3 | In-Out |
|30 | 2 | 1 | 2 | 1 | Other |
|31 | 2 | 1 | 2 | 2 | In-Out |
|32 | 2 | 1 | 2 | 3 | In-Out |
|33 | 2 | 1 | 3 | 1 | Other |
|34 | 2 | 1 | 3 | 2 | Other |
|35 | 2 | 1 | 3 | 3 | In-Out |
|36 | 2 | 1 | 3 | 4 | In-Out |
Appendix A: Theoretical permutations of tube actuation. (CONT)

| ID | Tube Region | Classification |
|----|-------------|----------------|
| I  | II          | III            | IV  |
| 37 | 2           | 1              | 4   | 3   | Other |
| 38 | 2           | 2              | 1   | 1   | Down  |
| 39 | 2           | 2              | 1   | 2   | In-Out|
| 40 | 2           | 2              | 1   | 3   | In-Out|
| 41 | 2           | 2              | 2   | 1   | Down  |
| 42 | 2           | 2              | 3   | 1   | Out-In|
| 43 | 2           | 3              | 1   | 1   | Out-In|
| 44 | 2           | 3              | 1   | 2   | Other |
| 45 | 2           | 3              | 1   | 3   | Other |
| 46 | 2           | 3              | 1   | 4   | Other |
| 47 | 2           | 3              | 2   | 1   | Out-In|
| 48 | 2           | 3              | 3   | 1   | Out-In|
| 49 | 2           | 3              | 4   | 1   | Out-In|
| 50 | 2           | 4              | 1   | 3   | Other |
| 51 | 2           | 4              | 3   | 1   | Out-In|
| 52 | 3           | 1              | 1   | 2   | In-Out|
| 53 | 3           | 1              | 2   | 1   | Other |
| 54 | 3           | 1              | 2   | 2   | In-Out|
| 55 | 3           | 1              | 2   | 3   | In-Out|
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| 56 | 3 | 1 | 2 | 4 | In-Out |
| 57 | 3 | 1 | 3 | 2 | Other  |
| 58 | 3 | 1 | 4 | 2 | Other  |
| 59 | 3 | 2 | 1 | 1 | Down   |
| 60 | 3 | 2 | 1 | 2 | In-Out |
| 61 | 3 | 2 | 1 | 3 | In-Out |
| 62 | 3 | 2 | 1 | 4 | In-Out |
| 63 | 3 | 2 | 2 | 1 | Down   |
| 64 | 3 | 2 | 3 | 1 | Other  |
| 65 | 3 | 2 | 4 | 1 | Other  |
| 66 | 3 | 3 | 1 | 2 | In-Out |
| 67 | 3 | 3 | 2 | 1 | Down   |
| 68 | 3 | 4 | 1 | 2 | Other  |
| 69 | 3 | 4 | 2 | 1 | Out-In |
| 70 | 4 | 1 | 2 | 3 | In-Out |
| 71 | 4 | 1 | 3 | 2 | Other  |
| 72 | 4 | 2 | 1 | 3 | In-Out |
| 73 | 4 | 2 | 3 | 1 | Other  |
| 74 | 4 | 3 | 1 | 2 | In-Out |
| 75 | 4 | 3 | 2 | 1 | Down   |
Determination of Uncertainty in Visual Identification of Events

Synchrotron x-ray phase contrast imaging has provided researchers and scientists with a window through which to view the in vivo mechanics of insect respiration [1]. Although synchrotron phase contrast imaging enables many internal structures to be visualized, as with any imaging technique, the resulting images are far from perfect. Tracheal structures in insects are highly amenable to visualizing because of the large contrast between the density of air and the density of tissue, but the boundary of the tracheal wall can sometimes be difficult to discern due to noise in the image and image blur from the moving wall during a dynamic tracheal compression cycle. Changes in effective tissue density along with the frequent pixilation of physiological boundaries impose limitations on the efficacy of tracking of the tracheal wall during tracheal compression. Manual digitization provides a quantitative estimate of the location of the wall, but it is time-consuming. We chose to identify phases of tracheal compression by frame-by-frame visual analysis in ImageJ [2]. Here, we compare these methods by applying both manual digitization and visual identification to ten compression cycles and quantifying the uncertainty.

Manual Digitization

Figure S1: Manual digitization of a tracheal tube using ImageJ and the Point Picker plugin. The trachea runs horizontally, and the two wall edges can be seen as dark lines.

Data Acquisition

Synchrotron x-ray video files were imported into ImageJ, and contrast and levels were adjusted to enhance the visibility of the tracheal tube walls [1]. The ‘Point Picker’ plugin was
used to place points on the images. Two points about 25 pixels apart were selected on the upper edge of the tracheal tube. A length of roughly 25 pixels was chosen because it allowed the digitized portion of tube wall to remain relatively straight regardless of broader curvature of the tracheal tube. Eight points were chosen at even intervals between the two initial points, yielding a total of ten data points along the top edge of the tube.

Two points were placed on the lower edge of the tube directly opposite the boundary points on the top edge so that they reflected orthogonally across the tube diameter. Eight points were spaced evenly between the two boundary points along the lower edge for a grand total of twenty data points. Figure S1 is a screenshot from ImageJ showing a typical example of point placement.

The x,y coordinates of the points were imported into Matlab, and linear regression was used to fit curves to the top and bottom boundaries of the tube. A perpendicular bisector was superimposed on the bottom boundary, and the distance between the intersection with the top
and bottom tube edges was defined as the tracheal tube diameter. The process was repeated for
each frame of the tracheal compression cycle.

Data Processing

A smoothing spline was fit to a plot of tracheal tube diameter versus frame number.
Numerical differentiation was used to solve for the zero slope point to find the transition between
rhythmic tracheal compression phases. The zero slope point of the derivative of tube diameter
occurs when the diameter has stopped changing, providing means to identify the transitions
between RTC phases: collapse-start, collapse-end, reinflation-start, and reinflation-end. If the
zero slope point occurred between two frames, the greater frame number was selected as the true
frame of the event. The frame number calculated using this technique was considered to be the
true frame of incidence for a specified event.

Visual Event Extraction

Data Acquisition

Video sequences were imported into ImageJ and visually scanned for transitions between
RTC phases. The researchers’ best estimate of the frame denoting each event (collapse-start,
collapse-end, reinflation-start, and reinflation-stop) was recorded.

Uncertainty Analysis

The drawback associated with visual data extraction is that accuracy depends on the
ability of the researcher to discern visually finite differences in tube movement and position,
which may vary between individual researchers.
Two sources of error were considered when calculating the total uncertainty ascribed to the events marking the transitions between RTC phases. The first source of error derives from the visual extraction of events. The events in five compression cycles from two image sequences of perceptively different quality were visually extracted. The process was repeated three times with a minimum of twelve hours between sessions over the course of several days. The data for the two image sequences were pooled to determine the standard error of repeated visual measurements.

The two image sequences were manually digitized using the protocol described above. The results of the manual digitization were considered as a benchmark to which the visual extraction results were compared. The uncertainty between the two techniques was calculated, accounting for error associated with visually identifying the true frame number of an event. The total uncertainty for each event was calculated to be the Euclidean norm of the two constituent uncertainties taken at 95% confidence. For collapse-start, collapse-stop, reinflation-start and reinflation-stop, the respective uncertainties were calculated to be $+/- 1.1$ frames, $+/- 1.6$ frames, $+/- 1.2$ frames and $+/- 3.2$ frames, corresponding to absolute durations of 0.04, 0.05, 0.04, and 0.11 s, respectively.

References

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