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An easy, cheap computerized method to assess two-dimensional trajectory parameters

Marie-Claire Cammaerts[1,*], Frederic Morel[2], Fabian Martino[2] & Nadine Warzée[2]

[1] Faculté des Sciences CP 160/12.
[2] Faculté des Sciences Appliquées, Service LISA CP 165/57, Université Libre de Bruxelles, 50, Av. F. Roosevelt, B-1050 Bruxelles, Belgium.

* Corresponding author: mtricot@ulb.ac.be

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Movement is essential for the survival of mobile organisms. Its study can help to determine taxonomic status (1), to isolate pheromones (2) and to understand biological mechanisms (3). It can also provide information on the health, physiological state and motivation of animals. However, it has rarely been rigorously quantified. We devised a manual method in 1973 (4) and computerized it in 1991 (5) but, despite its continuing use (e.g. 6), this processing became obsolete due to the evolution of computers. Plenty of modern programs exist (7 and references therein, 8, 9 and references therein) but require expensive equipment, take a long time and are generally appropriate for only one kind of assessment. We developed a user-friendly, cheap method that allows simultaneous assessment of orientation, linear speed and angular speed of any moving agent.

This software was tested on the ant *Myrmica rubra*, in a colony being maintained in the laboratory (Fig. 1A). Stimuli presented to the foragers were pieces (1 cm^2) of white paper and isolated heads of congeners, which emit the species' alarm pheromone.

Ant trajectories were manually recorded, using a water-proof marker pen, on a glass slide set over the ants' foraging area. They were then traced onto transparent polyvinyl sheets, which stuck to the screen of any PC (Fig. 1B). The trajectories could then be analyzed using the newly elaborated software installed on the PC:

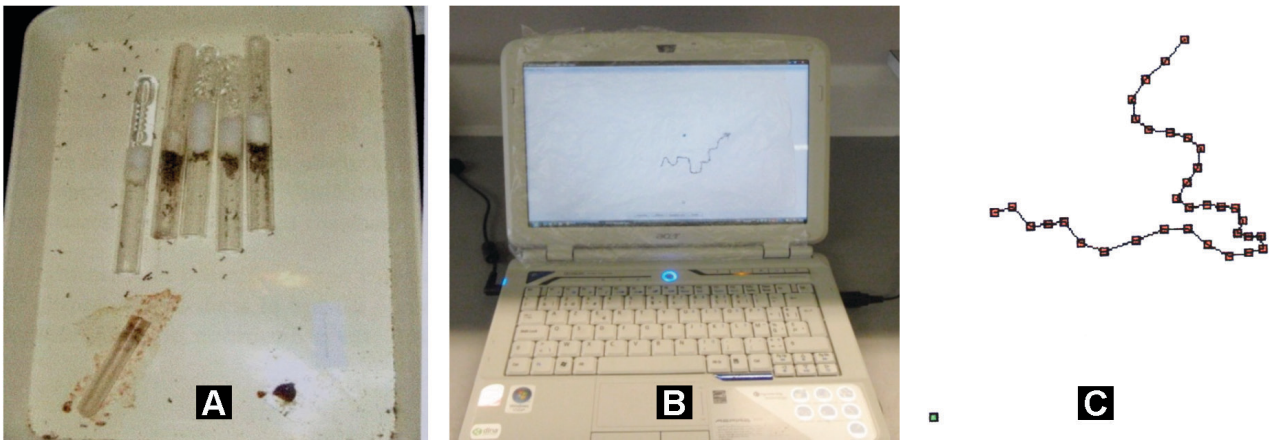
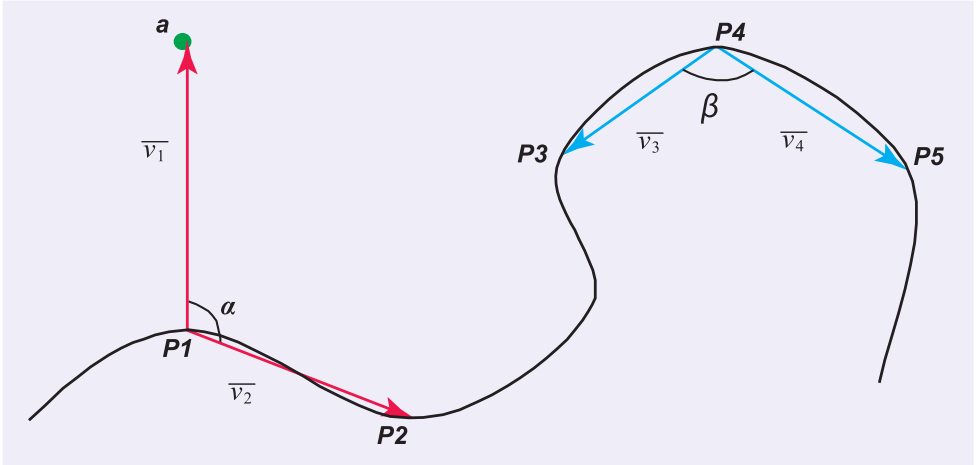


Fig. 1. – Three steps in the computerized analysis of trajectories. **A**: ants are kept in the laboratory in artificial nests. Trajectories are recorded on a glass slide set above the ants' tray and are then traced on a polyvinyl sheet. **B**: this sheet is stuck to the screen of a PC. Each trajectory is entered using a mouse. **C**: the updated software visualizes each trajectory and quantifies its orientation, linear and angular speed.

1. The distance between two points on the screen, initially assessed in pixels, is converted into a metric unit using a dialog box, for both the X- and Y-axes.
2. The successive points of the trajectory are entered by clicking with the mouse, which visualizes, in red, the trajectory on the screen (Fig. 1C). The point towards which the moving agent was expected to go is then located, in green, on the screen (Fig. 1C).
3. The user then states that the trajectory entering is finished and, after that, he/she enters, in a window, the total time spent by the moving agent to move along its trajectory.
4. Validating the last operation starts the calculation, by the newly-elaborated software, of the three following variables (Fig. 2). The orientation (O) of the moving agent towards a given point of the environment is the sum of the angles, measured at each successive point of the registered trajectory, made by the segment 'point i of the trajectory – given point' and the segment 'point i – point i + 1' divided by the number of measured angles. This variable can be measured in angular degrees, for instance. The linear speed (V) of the agent is the length of its trajectory divided by the time spent moving along this trajectory. It can be measured in mm/s, for instance. The angular speed (S) (i.e. the sinuosity) of the animal's trajectory is the sum of the angles, measured at each successive point of the trajectory, made by the segment 'point i – point i – 1' and the segment 'point i – point i + 1', divided by the length of the trajectory. This variable can be measured in angular degrees/cm, for instance.
5. The required calculated values appear on the screen of the PC, the entire operation lasting 20-30 sec. The user can then 'shut the program' or 'begin again', directly entering a new trajectory.



a = a given point in the environment

V: distance / time

$$\mathbf{O:}\begin{cases}\overline{v_1} = (a_x - p1_x, a_y - p1_y)\\ \overline{v_2} = (p2_x - p1_x, p2_y - p1_y)\end{cases} \qquad \mathbf{S:}\begin{cases}\overline{v_3} = (p3_x - p4_x, p3_y - p4_y)\\ \overline{v_4} = (p5_x - p4_x, p5_y - p4_y)\end{cases}$$

$$\overline{v_1}\cdot\overline{v_2} = \|\overline{v_1}\|\|\overline{v_2}\|\cos\alpha \qquad \overline{v_3}\cdot\overline{v_4} = \|\overline{v_3}\|\|\overline{v_4}\|\cos\beta$$

$$\rightarrow \alpha = \cos^{-1}\left(\overline{v_1}\cdot\overline{v_2}\,/\,\|\overline{v_1}\|\|\overline{v_2}\|\right) \qquad \rightarrow \beta = \cos^{-1}\left(\overline{v_3}\cdot\overline{v_4}\,/\,\|\overline{v_3}\|\|\overline{v_4}\|\right)$$

$$O = \sum_i \alpha_i / number\ of\ angles \qquad S = \sum_i \beta_i / distance$$

Fig. 2. – Mathematical reasoning underlying the quantification of the orientation (**O**), linear speed (**V**) and angular speed (**S**) of a trajectory. The three variables are defined in the text.

Table 1

Comparison of the manual **(M)** and the computerized **(L)** method. Ten ant trajectories obtained in the presence of a blank paper (control) and of an isolated congener's head (test) were analyzed and the difference between the two methods was evaluated. Differences are less than the experimental errors. O=orientation (angular degrees), V=linear speed (mm/sec), S=angular speed (angular degrees/cm).

Control											mean	difference
O	**M**	74.6	124.2	101.9	119.3	105.0	114.0	86.8	106.1	57.3	101.1	99.8	6.5%
	L	70.4	113.8	98.3	118.1	91.5	109.7	89.9	101.6	44.5	97.2	93.5	
V	**M**	11.0	7.0	8.0	8.0	12.5	10.0	11.5	9.0	10.0	10.0	9.6	12%
	L	11.6	6.8	10.3	6.0	10.8	10.6	9.5	10.1	9.3	10.3	8.5	
S	**M**	137	156	183	63	138	138	155	253	119	214	162	5%
	L	138	149	182	62	153	149	166	225	113	201	154	

Test											mean	difference
O	**M**	53.3	38.0	46.0	39.2	33.3	42.0	60.9	20.0	60.0	31.3	42.4	4.6%
	L	55.2	36.2	43.3	37.0	31.5	44.9	64.3	17.2	52.3	22.9	40.5	
V	**M**	12.0	16.0	18.0	20.0	18.0	22.0	24.0	17.0	24.0	18.0	18.9	3%
	L	13.4	15.4	18.2	17.2	16.6	19.2	21.9	16.5	26.7	17.7	18.3	
S	**M**	109	100	106	109	106	99	115	145	105	157	115.1	0.6%
	L	93	109	73	126	87	111	122	150	105	183	115.8	

Table 2

Assessment of the linear (V) and angular speed (S) of *Tribolium castaneum* and of *Paramecium caudatum* under control and experimental conditions. *T. castaneum* was observed directly, like the ants, while *P. caudatum* was observed under a stereomicroscope (Mag. = 23 X), this requiring a unit adaptation. N = number of individuals observed; results of non-parametric χ^2 tests between control and experiments: P= level of probability; NS = difference not significant at P = 0.05. An activated GSM had an impact on the observed animals.

T. castaneum	N	V (mm / sec)	S (angular degrees / cm)
Control	42	5.2 (4.6 - 5.8)	150 (120 - 183)
+ GSM on	31	3.8 (3.2 - 4.4) P < 0.001	398 (343 - 469) P < 0.001
+ GSM off	29	5.1 (4.7 - 6.2) NS	174 (145 - 220) NS
P. caudatum	N	V (mm / sec)	S (angular degrees / mm)
Control	23	0.63 (0.57- 0.67)	179 (138 - 200)
+ GSM on	34	0.50 (0.39 - 0.58) P < 0.001	465 (340 - 534) P < 0.001
+ GSM off	32	0.66 (0.59 - 0.74) NS	172 (117 - 196) NS

The manual and the computerized methods give identical results (Table 1), but the computerized one is 30 times faster and therefore allows analysis of many more trajectories, and is more precise, human errors being avoided.

The newly-computerized method was then used to make five assessments, and was thus tested.

1. Trajectories of the beetle *Tribolium castaneum* were successfully analyzed under normal conditions, near a switched-on mobile phone (GSM) and near a switched-off GSM (Table 2). The new method is particularly applicable to small moving animals. Note the effect of an activated GSM on the insects' movement.
2. Trajectories of the protozoan *Paramecium caudatum* were analyzed under normal conditions, near a switched-on GSM and near a switched-off GSM (Table 2). A camera lucida was applied to the stereomicroscope under which *P. caudatum* were set. The new method allows analysis of the movement of any microscopic agent in this manner. Note, once more, the effect of an electromagnetic field on living organisms.
3. Pieces of white paper (1 cm^2) were deposited for 8 days on ant cemeteries (Fig. 3A) and were then presented to foragers whose movement was analyzed using the described method (Table 3). The foragers were not attracted by the papers but their angular speed considerably increased. Ants transporting corpses move thus randomly away from their nest and in a sinuous increasingly slowing-down pattern as they come nearer to a cemetery. They finally stop there and drop the corpses. While returning then to their nest, they deposit their trail pheromone along a short distance (personal observation), which explains the ethological effect of cemetery sites on the ants. The new computerized method thus provided, in a few minutes, an explanation for the presence of ant cemeteries, on given places, far from the nests.
4. *Myrmica* ants are attracted by their specific alarm pheromone contained in the head of workers (Table 4). Cross-tests using isolated heads of known and unknown ants (f.i. newly collected) (Fig. 3B) followed by analysis of the numerous recorded ant trajectories enable recognition of an unknown (f.i. collected) species. Such a long process can be efficiently performed only by using this rapid computerized method. Such taxonomic recognition of closely related species can be

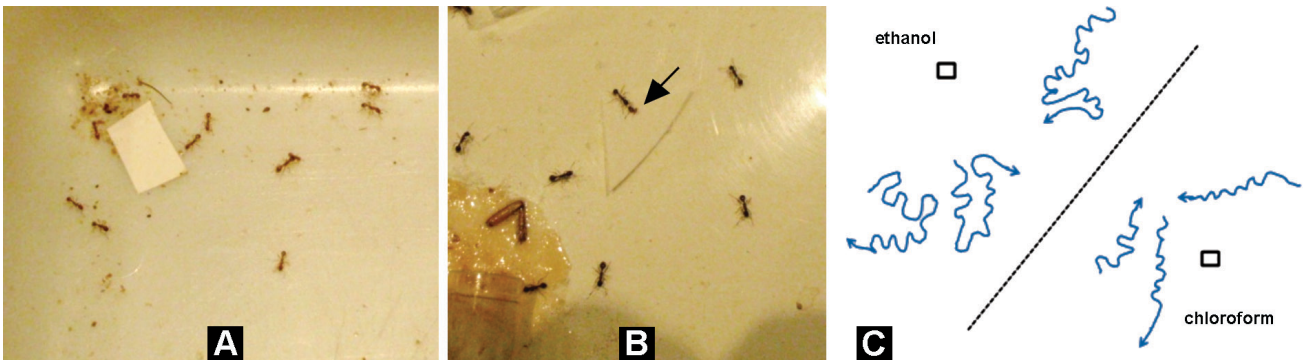


Fig. 3. – Three illustrated uses of the method. **A:** pieces of paper were deposited on ant cemeteries and then presented to foragers. They were not attractive to the ants but decreased their angular speed. They thus may be impregnated with trail pheromone deposited by ants leaving the cemeteries sites. **B:** isolated heads of three ant species were presented to foragers of these species to see if such cross tests can help recognizing unknown species. Here, the head of an individual of *M. sabuleti* (pointed by an arrow) is presented to workers of *M. rubra*, which are not attracted by the non-specific stimulus. Cross tests and assessments using our method can thus help discriminating between species. **C:** trajectories of ants moving near a small amount of ethanol or chloroform. Ethanol increased the ants' linear and angular speed while chloroform decreased their linear speed. Simple ethological tests together with our software-based method can help detect minute amounts of drugs in samples.

Table 3

Locomotion of *Myrmica sabuleti* foragers in front of their cemeteries. Blank pieces of paper or paper deposited for 8 days at cemeteries were presented to foragers. The orientation towards the paper (angular degrees), the linear speed (mm/sec) and the angular speed (angular degrees/cm) of 60 or 30 (= N) foragers were assessed using our software. The distributions of the values obtained for each two stimuli were compared using the non-parametric χ^2 test. P = level of probability; NS = difference not significant at P = 0.05.

variable	untreated paper	paper deposited at cemeteries	statistics
Orientation	N = 60 89.3 (66.6 – 105.3)	N = 30 91.1 (75.7 – 107.0)	NS
Linear speed	N = 60 12.8 (11.8 – 14.7)	N = 30 11.1 (9.2 – 14.8)	P < 0.05
Angular speed	N = 60 183 (147 – 211)	N = 30 223 (211 – 245)	P < 0.001

Table 4

Cross-tests between three *Myrmica* species, using isolated worker heads presented to foragers. The orientation (O; angular degrees) towards the head, the linear speed (V; mm/sec) and the angular speed (S; angular degrees/cm) of 10 foragers were assessed each time, using our software. Ants clearly oriented themselves only towards isolated heads of their own species. Cross-tests, together with our computerized method, are thus helpful for taxonomic purposes.

Species whose head was presented	Tested species *M. rubra*	*M. ruginodis*	*M. sabuleti*
Myrmica rubra	O 44.7(42.5-52.0) V 24.6(24.1-26.3) S 77 (75-80)	O 105.5(86.2-119.8) V 19.3(16.8-20.6) S 91 (76-106)	O 81.4(69.5-96.7) V 17.2(16.2-17.9) S 130 (121-150)
Myrmica ruginodis	O 82.8(76.9-89.7) V 19.6(18.9-20.8) S 116 (111-148)	O 34.1(31.4-46.0) V 29.8(27.6-32.8) S 79 (63-91)	O 93.9(82.9-101.5) V 22.5(20.2-24.9) S 116 (110-127)
Myrmica sabuleti	O 107.2(95.7-118.2) V 19.6(17.6-22.5) S 160 (147-170)	O 93.6(74.8-118.2) V 20.0(17.3-20.7) S 121 (97- 134)	O 44.4(35.1-57.3) V 22.8(19.3-24.1) S 126 (106-143)

Table 5

Response of *Myrmica sabuleti* workers to ethanol and chloroform. 10 µl of differently-concentrated solutions of these substances were presented to foragers and the locomotion of 10 of them was assessed using the here related software. The concentration (%) is given in the first column; the quantity (µl) presented, in the second one. O = orientation towards the stimulus, angular degrees; V = linear speed, mm/sec; S = angular speed, angular degrees/cm. χ^2 tests between results for 'pure water' and 'substances': P = level of probability, * = P < 0.05 or 0.02, ** = P < 0.001, otherwise = result non significant at P = 0.05.

Concentration	Quantity	O	V	S
Pure water		66.3(61.2-71.7)	14.5(13.7-15.7)	142(133-153)
Ethanol				
0.001	0.0001	80.9(70.3-108.8)	18.0(15.6-21.2) *	167(154-194) *
0.01	0.001	78.8(68.3-89.3)	20.5(18.1-22.1) **	194(181-203) **
0.1	0.01	81.1(61.7-105.8)	21.3(16.7-23.2) **	221(213-232) **
1	0.1	101.3(69.5-109.7)	24.6(21.6-26.9) **	216(197-241) **
10	1	77.9(67.3-91.8)	25.4(22.3-28.3) **	218(204-228) **
Chloroform				
0.0001	0.00001	77.3(60.7-93.2)	12.8(12.2-14.2)	190(173-224) **
0.001	0.0001	97.4(87.9-111.2)	9.7(7.3-11.9) **	212(161-239) **
0.01	0.001	88.5(80.4-102.0)	8.8(8.1-9.2) **	279(266-291) **
0.1	0.01	85.1(65.2-99.2)	8.1(6.8-9.8) **	275(223-297) **

extended to any animals that have specific pheromonal secretions. It can be used as an aid to morphological or genetic determination. This technique should be applied, for instance, to related bumblebee species (10), virgin females responding only to the pheromonal secretion of conspecific males.

5. *Myrmica* ants react to ethanol by increasing their linear and angular speed (Fig. 3C), and do so down to 0.0001 µl of ethanol, which corresponds to an aqueous solution of 0.001% (Table 5). These ants also react to chloroform, but by decreasing their linear speed (Fig. 3C), this occurring down to a presentation of 0.00001 µl of chloroform, e.g. an aqueous solution of 0.0001%. For revealing these kinetic reactions, many trajectories must be analyzed, and this can be done, in a short time, only by using this rapid, simple method. So, using this method, *Myrmica* ants can be used to detect small amount of any given drugs in collected material.

In conclusion, RODUIT (11) wrote: 'no universal solution exists for the analysis of trajectories'. This is true when the solution requires highly technical equipment, sophisticated software and many conditions for being used. On the contrary, a simple method – requiring cheap material, easy-to-use software and having no conditions for being used – may be universal or, at least, used in a first step to check if it may be promising to use more onerous methods. The user-friendly system we have here related is such a simple method. It requires no program license and can be used by many persons at the same time. It is thus competitive with other more sophisticated methods. The software, labeled OVS, will be available on the website of the journal as soon as the present paper is published.

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