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RESEARCH ARTICLE

Effect of interactions among individuals on the chemotaxis behaviours of *Caenorhabditis elegans*

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ABSTRACT

In many species, individual social animals interact with others in their group and change their collective behaviours. For the solitary nematode *Caenorhabditis elegans* strain N2, previous research suggests that individuals can change the behaviour of other worms via pheromones and mechanosensory interactions. In particular, pheromones affect foraging behaviour, so that the chemotactic behaviours of individuals in a group (population) can be modulated by interactions with other individuals in the population. To investigate this, we directly compared the chemotactic behaviours of isolated (single) worms with those of individual animals within a population. We found that worms approached an odour source in a distinct manner depending on whether they were alone or in a population. Analysis of behaviours of the N2 worm and a pheromone production-defective mutant revealed that the 'pirouette' strategy was modulated by interaction of the worms via pheromones. Thus, pheromones play an important role in the characteristic collective behaviours seen in the population condition.

KEY WORDS: Collective behaviour, Olfactory, Pheromone, Trail, Contact

INTRODUCTION

Individual animals interact with others in their group, leading to changes in collective behaviour. The collective behaviours of swarming ants, schooling fish and flocking birds in groups of social animals have previously been studied (Sumpter, 2006; Visscher, 2007; Couzin, 2009; Herbert-Read, 2016). Recently, *Drosophila melanogaster*, which is classified as a solitary species, was also shown to exhibit collective behaviours driven by mechanosensory interactions (Ramdya et al., 2015). Although the laboratory nematode *Caenorhabditis elegans* strain N2, which has acquired a gain-of-function phenotype through modification of the *npr-1* neuropeptide receptor, is a solitary species (de Bono and Bargmann, 1998; Rockman and Kruglyak, 2009; Weber et al., 2010), population density regulates reproductive development and dauer (*C. elegans* larvae arrested at the second moult) formation via pheromones (Ludewig and Schroeder, 2013). Thus, like *D. melanogaster*, worms may affect each other's behaviours. In fact, pheromone signalling affects olfactory adaptation (Yamada et al., 2010), and some ascaroside pheromones regulate exploratory foraging in *C. elegans* (Greene et al., 2016a,b). Moreover, physical contact has been shown to influence collective behaviours; swimming behaviour synchronizes as two worms approach each other (Yuan et al., 2014). Taken together, chemotactic behaviours in populations are inevitably represented as collective behaviours, which include crucial roles in competition for resources. Although factors that have an important influence on collective behaviours in *C. elegans* have been identified, there has been no research directly comparing the chemotactic behaviours of isolated worms with those of individual animals from populations.

Several studies on *C. elegans* describe chemotactic behaviours in single animals (Pierce-Shimomura et al., 1999; Iino and Yoshida, 2009; Wakabayashi et al., 2015; Yamazoe-Umemoto et al., 2015), namely the pirouette and weathervane strategies. In the pirouette strategy, worms show directional changes with sharp turns (pirouettes) when they detect a negative temporal change in the odour concentration ($dC/dt<0$) (Pierce-Shimomura et al., 1999). In the weathervane strategy, animals gradually move to higher concentration regions (Iino and Yoshida, 2009). Although these methods have mainly been used to investigate the behaviour of isolated worms, it is unclear whether the behaviour of *C. elegans* differs depending on interactions with other worms.

Here, we compared the behaviour of *C. elegans* in a chemotaxis assay under different collective conditions. To investigate the trajectories under each condition, we showed that worms approached an odour source in different manners based on their interaction with other worms. In addition, by using mutants with defective production of pheromones, we demonstrated that pheromones were necessary for the collective behaviour shown in the population condition.

MATERIALS AND METHODS

Strains

All strains were cultured at 20°C on nematode growth medium (NGM) plates with *Escherichia coli* OP50 (Brenner, 1974). The strains were hermaphrodite N2, which is used as a wild-type strain in the laboratory, and hermaphrodite *daf-22* (m130) II mutants, which are defective in producing one group of pheromones, the ascarosides.

Chemotaxis assay

We designed three conditions for the chemotaxis assay: single, population and paired (Fig. 1A,B). The assay plate consisted of 8 ml of 1.8% agar, 1 mmol l^{-1} $CaCl_2$, 1 mmol l^{-1} $MgSO_4$ and 5 mmol l^{-1} KH_2PO_4 in a 10 cm Petri dish (ThermoFisher Scientific, Waltham, MA, USA). In all experiments, some worms were moved into S-basal buffer in a microtube with a sterilized platinum wire, and were washed. Then, we transferred all of the worms to an assay plate with buffer to enable us to pick each worm in the subsequent procedure easily. For each assay, eight worms in

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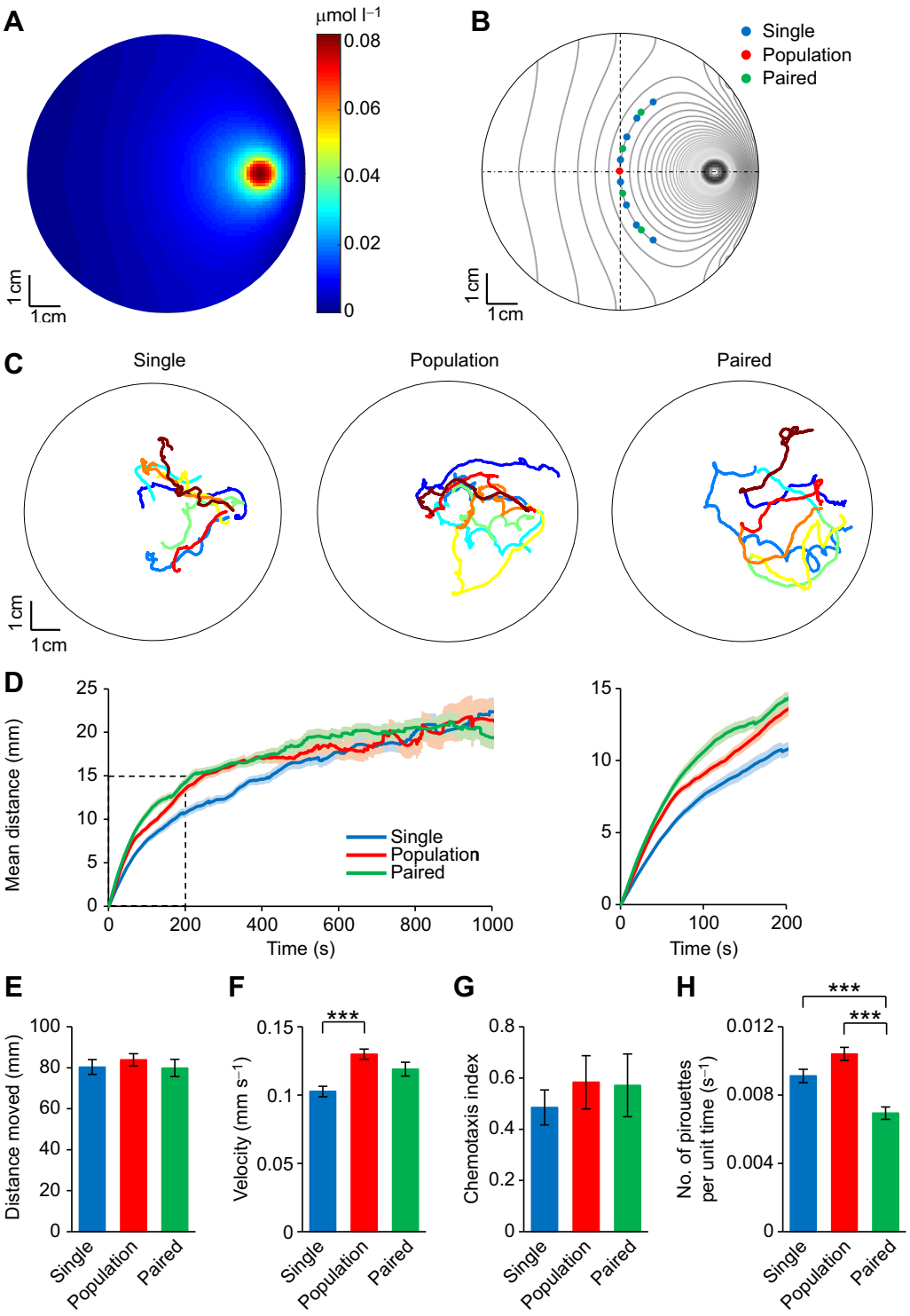

Fig. 1. The chemotaxis behaviour of worms in collective conditions. (A) Representation of the odour concentration on the assay plate. (B) Start positions of worms in each condition. Each point indicates the place where worms were spotted (see Materials and Methods). Contour lines indicate the odour concentration. (C) Representative results for each condition. Each animal is represented by a different colour. (D) Mean distance between individual worms. Traces indicate the mean (with shading representing the s.e.m.) distance between neighbouring worms. The right graph is an enlargement of the area enclosed by the dashed line in the left graph. (E–H) Mean (±s.e.m.) distance moved (E), velocity (F), chemotaxis index (G) and number of pirouettes per unit time (H) (N=12, n=93, 96 and 95, respectively, for single, population and paired conditions; N, n indicate the number of assays and animals, respectively). E,H: Mann–Whitney U-test with Bonferroni correction; F,G: Student's t-test with Bonferroni correction; ***P<0.001.

total were moved to start points on another assay plate where 2.5 µl (single and paired) or 4 µl (population) of distilled water had been spotted in advance (Fig. 1B). The number of animals in each spot was as follows: single, 1; population, 8; paired, 2. Any excess distilled water was immediately removed with Kimwipes. The attractive odour for the assay was 1 µl of 10^{-2} dilution of isoamyl alcohol (IAA) in ethanol (EtOH), which was spotted onto the plate; 1 µl of EtOH was also spotted onto the other side of the plate. To each spot, 500 mmol l^{-1} of sodium azide (an anaesthetic) was applied in advance so that animals were restrained once they reached the odour spot. The total time for the chemotaxis assay was 30 min.

Image acquisition and analysis

We captured images (1080×1080 pixels, 0.09 mm per pixel) with a web camera (HD Pro Webcam C920, Logitech, Lausanne, Switzerland) fixed on a stand, every second for 30 min using a custom-written program in MATLAB 2016a (MathWorks, Natick, MA, USA). An assay plate was set on an A4-sized LED light source

(1-2785-11, AS ONE Corporation, Osaka, Japan) with the lid on the bottom. To ensure that the images of the transparent animals were clear in dark field, a sheet of black paper was placed on the lid.

For analysis, we initially obtained the tracks of the animals using a modified parallel worm tracker in MATLAB (Ramot et al., 2008). In the original program, the tracks of each worm were sometimes distorted as a result of noise or collisions of the animals. Here, we aimed to track the trajectory of each animal from start to finish, so we complemented some parts of the trajectories and combined fragments of trajectories manually. Instances where worms hardly moved from their start point were discarded from further analyses; the number of animals affected (number of immobile worms/total number of worms) was as follows: N2 single 3/96, population 0/96, paired 1/96; *daf-22* mutants single 6/96, population 1/96, paired 2/96. In addition, if worms arrived at the high odour concentration area, their subsequent trajectories were removed from the dataset because the animals were immobilized by sodium azide. For worms that reached the edge of plates, the trajectory data before arrival at the edge were used for analysis because most animals climbed the side wall after that. Before the analysis, x and y positions were smoothed by a median filter every five time points (5 s). Distances between individual worms were calculated as the mean Euclidian distance from each worm to all other worms at a particular time. For the single and paired conditions, the distances were calculated after the start points of each animal were translocated to the centre of the plate and rotated (Fig. S1), because otherwise the start points of individual animals were different in these conditions. In brief, the distances in the single and paired conditions were estimated based on the assumption that worms had started from the centre like those in the population condition. The chemotaxis index was calculated as follows: [(number of animals within a 1 cm radius of the odour spot) – (number of animals outside this area)]/total number of animals on the plate.

The behaviours of worms are so simple that the trajectories were analysed following conventional methods (Pierce-Shimomura et al., 1999; Iino and Yoshida, 2009; Wakabayashi et al., 2015; Yamazoe-Umemoto et al., 2015). Behaviours were categorized into three groups: run, pirouette and immobilized. The following analysis of pirouette behaviours is explained in detail elsewhere (Pierce-Shimomura et al., 1999). The pirouette behaviour includes sharp turns and subsequent short-term migrations. In our experiments, sharp turns were defined as instances where the absolute turning rate ($|\mathrm{d}\theta|/\mathrm{d}t$) was over 90 deg. Angle changes ($\mathrm{d}\theta$) were differences in direction before and after specific time points. Using this criterion, behaviours were classified as sharp turns and migrations. The distribution of migrations was fitted by the sum of two exponentials, to give the critical migration duration t_{crit} (Pierce-Shimomura et al., 1999). According to our preliminary experiment, this duration was calculated to be t_{crit}=12.92 s (data not shown). Therefore, animals that had migration durations of less than t_{crit} and sharp turns were designated as having performed a pirouette, while those for which the migration duration was longer were classified as showing run behaviour. In addition, when worm velocity was <0.01 mm s^{-1} in half of 10 sequential time points, worms were designated as immobilized.

We calculated the pirouette initiation rate as follows. First, we calculated the initiation rate for each specific term. Data were fitted with the sigmoid function:

$$P_{\mathrm{pirouette}} = \frac{\beta}{1 + e^{\alpha \times (\mathrm{d}C/\mathrm{d}t)}} + \delta, \quad (1)$$

where $P_{\mathrm{pirouette}}$ is the pirouette initiation rate against the time derivative of the odour concentration ($\mathrm{d}C/\mathrm{d}t$), and α, β and δ are parameters determined by curve fitting with non-linear least-squares methods (MATLAB 2016a, MATLAB fit function with the NonlinearLeastSquares option). To analyse the effect of collisions between worms, we calculated the cosine of the directional vectors before and after a collision and the vectors to the odour source (Fig. S3B), which were defined as direction values and attracted values. In this case, a collision was designated if worms were less than 0.5 mm from one another. The directional vectors used here were the same as those used to calculate the curving rate, described below.

To analyse the weathervane strategy, we calculated the curving rate as previously described (Iino and Yoshida, 2009). The concentration gradient orthogonal to the worm's direction was obtained as follows. For each time point, we first identified the worm's direction and calculated the concentration gradient orthogonal to it. For analysis of worms crossing trails, we determined the point at which worms crossed the trails with a semi-automated program written in MATLAB. To obtain the probability of pirouettes occurring within 10 s of worms crossing the trail, the total number of pirouettes was divided by the total number of passing trails. The binominal test for this probability was conducted as follows. First, we examined the number of pirouettes occurring within 10 s of specific time points sampled randomly. The number of time points chosen for each worm was the mean number of worms crossing the trails in the experimental data. Then, we repeated this process 1000 times and obtained the mean probability of pirouettes within 10 s after random time points, as the hypothesized probability. The parameters were used in binominal tests and the results are presented in Table S2. The directions before and after worms crossed the trails were calculated every five time points (5 s) before and after the events (Fig. S5C,E). The direction of the trails was calculated from their trajectories. All statistical tests were performed in R (version 3.4.1, exactRankTests and kSamples libraries) except for regression analysis, which was conducted in Excel 2016 (Microsoft, Seattle, WA, USA).

Numerical estimation of odour gradient

To estimate the odour distribution on the agar surface, we used a numerical simulation written in C++. The concentration (C) of IAA and EtOH was calculated with a three-dimensional diffusion equation:

$$\frac{\partial C}{\partial t} = D\nabla^2 C. \quad (2)$$

The equation was solved with the second-order central difference method (Press et al., 1992) for 30 min. The diffusion coefficients in the air were D_{EtOH}=0.123 $\mathrm{cm}^2\ \mathrm{s}^{-1}$ and D_{IAA}=0.0692 $\mathrm{cm}^2\ \mathrm{s}^{-1}$ (Yaws, 2009a,b). The boundary condition for the gas–surface interface at specific odour spots was determined using methods described previously (Yamazoe-Umemoto et al., 2015) (Y. Iwasaki, personal communication):

$$-D\frac{\partial C}{\partial z} = \frac{E}{M}\left(\gamma(t)\chi(t) - \frac{C}{C_{\mathrm{sat}}}\right). \quad (3)$$

The evaporation rates per unit time and unit area were E_{EtOH}=1.98×10^{-11} g $\mathrm{cm}^{-2}\ \mathrm{s}^{-1}$ and E_{IAA}=0.127×10^{-11} g $\mathrm{cm}^{-2}\ \mathrm{s}^{-1}$, and the saturation concentrations were C_{satEtOH}=3.19 mmol l^{-1} and C_{satIAA}=34.5 µmol l^{-1} (ASTM D3539-87, 2004; Nylén and Sunderland, 1965).

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The activity coefficients were $\gamma_{EtOH}=\gamma_{IAA}=1$ (Ramsbotham, 1980). $\chi(t)$ is the molar fraction, and M is the molecular mass (EtOH, 46.07 g mol^{-1}; IAA, 88.148 g mol^{-1}). The initial radius of the odour spot was 6 mm. The Neumann boundary condition was used for the other surfaces including the wall and lid. The radius of the plate was 45 mm and the height was 10 mm. After simulation, spline interpolation (MATLAB fit function with the cubicinterp option) was used to calculate the odour concentration where the worm was located because the spatial mesh of the simulation was coarser than the measurement.

RESULTS

Interactions between individuals affect the trajectories of chemotaxis

To reveal whether interactions between worms affect chemotaxis, we examined chemotaxis of worms under two conditions: single and population. For the single condition, one worm was set at each starting point with even spacing and the same estimated odour concentration, while for the population condition, eight worms were placed at the same start point (Fig. 1B). Compared with worms in the population condition, those in the single condition were likely to move to the odour source directly (Fig. 1C). In contrast, it appeared that worms in the population condition dispersed away from their neighbours. To understand this quantitatively, we calculated the distances from each worm to the other worms. For the single and paired conditions (described below), the distances were estimated after the start point of each worm was transferred and superimposed to the centre of the plate (Fig. S1; see Materials and Methods). The distances between individual animals in the population condition increased more rapidly than those for worms in the single condition in the first 200 s (Fig. 1D), suggesting that the trajectories of chemotaxis differed between the single and population conditions.

Next, we examined whether the phenomenon described above could be caused by interactions between worms. To address this question, we used a paired condition in which two worms were placed a set distance apart at each starting point (Fig. 1B). In the paired condition, the trajectories also appeared to spread (Fig. 1C), and the distances between individual animals increased steeply, similar to measurements in the population condition (Fig. 1D), suggesting that the same phenomenon observed in the population condition also occurred in the paired condition. Therefore, these results indicate that interactions between two worms are an important factor affecting chemotactic behaviours.

Although worms in the population and paired conditions did not appear to move to the odour source directly from the start points compared with those in the single condition, the distance moved did not differ between conditions (Fig. 1E). Similarly, the attraction to odour (chemotaxis index) over 30 min was the same for all conditions (Fig. 1G). In contrast, worms in the single condition moved slower than those in the population condition (Fig. 1F). Based on these results, behaviours barely differed between conditions, but interactions between two worms appeared to alter their approach trajectories towards the odorant. This suggests that interactions between two or more worms can change the behavioural process used to reach the odour source.

Interactions affect pirouette strategies

Previous research has revealed that single worms use pirouettes (Pierce-Shimomura et al., 1999) and weathervane strategies (Iino and Yoshida, 2009) to approach attractants. Pirouettes, which include sharp turns and subsequent short migrations, allow worms to significantly change direction towards an odour source. Because worms in the population and paired conditions required large directional changes to move to the odour source, pirouettes may play an important role in interactions between worms. The probability of pirouettes in the population and paired conditions (collective conditions) differed from that in the single condition, but the population and paired conditions did not show any common tendencies (Fig. 1H; Fig. S2). To investigate this further, we evaluated the distribution of the time derivative of the odour concentration (dC/dt) at pirouette initiation (Fig. 2), as the occurrence of pirouettes depends on dC/dt (Pierce-Shimomura et al., 1999). The cumulative probability distribution in the single condition was steeper than that for the collective conditions (Fig. 2B, left and centre), and the distributions in the population and paired conditions showed a similar trend (Fig. 2B, right). These results indicate that worms in the single condition initiated pirouettes for smaller changes in odour concentration.

Pheromones are crucial for chemotaxis with interaction

According to the results of the collective conditions (Figs 1D and 2B), worms in the population and paired conditions showed similar trends in behaviour. Remarkably, worms appeared to avoid each other in the population and paired conditions (Fig. 1D); thus, interactions between two or more worms could change their chemotactic behaviours towards odorants. This could conceivably be due to pheromone signals and physical contact. Thus, we investigated whether pheromones altered behaviour between the single and paired conditions. We used the *daf-22* mutant in which ascaroside pheromones are not produced (Golden and Riddle, 1985; Jeong et al., 2005; Butcher et al., 2007; Pungaliya et al., 2009). As expected, *daf-22* mutants in the paired condition showed a similar tendency in chemotactic behaviours to those in the single condition (Fig. 3A). The difference between N2 in the single and paired conditions (Fig. 2) disappeared in the absence of pheromones (Fig. 3G,H), indicating that pheromones modulate the initiation of pirouettes by chemical stimuli. However, the results for the population condition did not conform to our expectations. Compared with the other conditions, *daf-22* mutants in the population condition spread out from the start point (Fig. 3A) and the parameters of chemotactic behaviour were significantly different (Fig. 3B–D). Remarkably, *daf-22* mutants in the population condition collided with each other more times than in the other conditions and versus the N2 strain (Fig. 3F; Fig. S3A). This suggested that physical contact was the cause of this tendency in *daf-22* mutants in the population condition. In fact, the cumulative probability slope for dC/dt at pirouette initiation in *daf-22* mutants in the population condition appeared to shift left and the inflection point was not around zero, suggesting that physical contact drove pirouettes regardless of odour concentration. Taken together, interactions between individual worms via pheromones change chemotactic behaviour by decreasing the temporal change of odour concentration required for pirouette initiation.

As shown above, excessive physical contact also changed the chemotaxis (Fig. 3, population condition). To investigate additional effects of physical contact in the other conditions, we evaluated behaviour before and after collision (Figs S3 and S4). For N2 worms, animals in the population condition collided more frequently than those in the other conditions (Fig. S3A). To understand whether physical contact changes behaviours, the running directions of worms to the odour source before and after collisions were calculated by defining the cosine of the running direction as the direction value (Fig. S3B; see Materials and Methods). If this value is close to +1, worms do not change their

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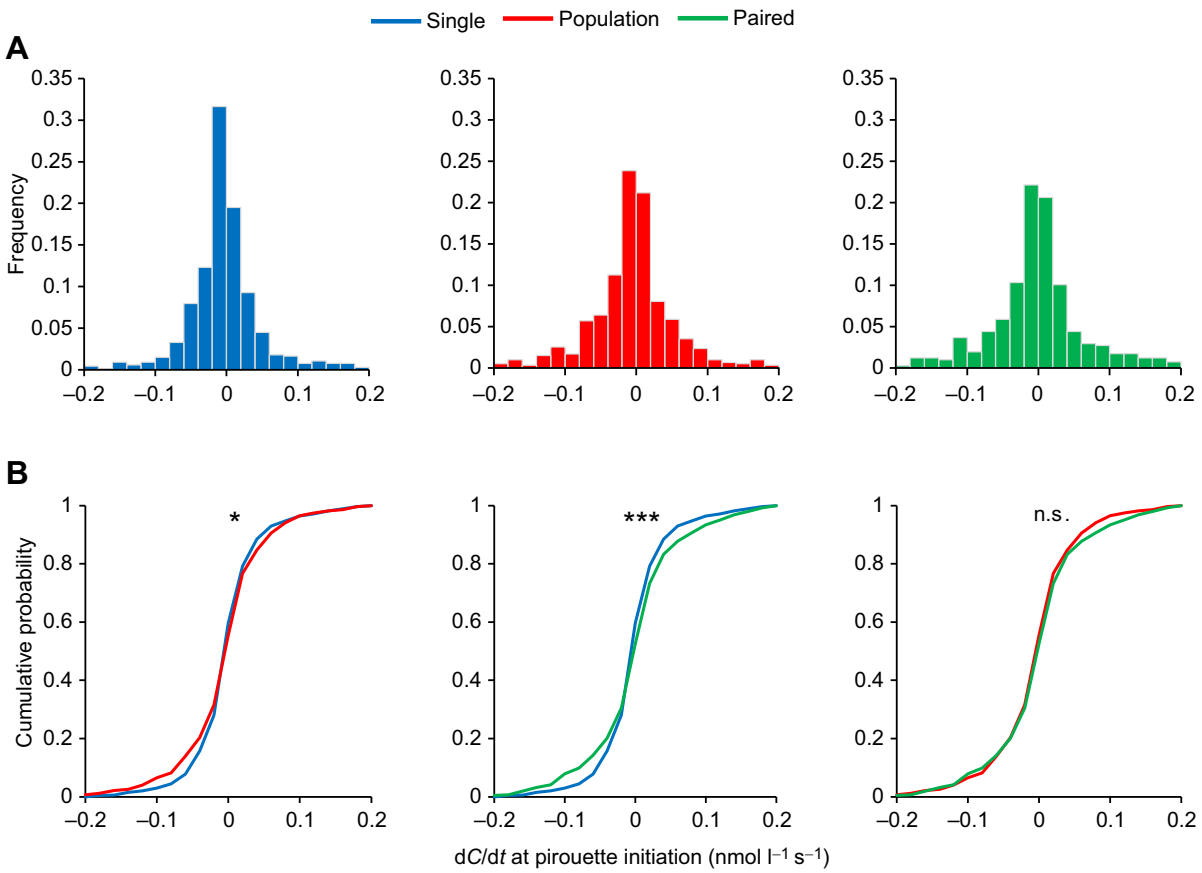

Fig. 2. Time derivative of the odour concentration (d*C*/d*t*) eliciting pirouette behaviours. (A) Histograms for commencement of pirouette behaviours in worms under each condition. (B) Cumulative probability of the frequencies in A: left, single and population; middle, single and paired; right, population and paired (*N*=12, *n*=93, 96 and 95, respectively, for single, population and paired conditions; *N*, *n* indicate the number of assays and animals, respectively). Non-parametric two-sample Anderson–Darling test with Bonferroni correction; *P<0.05, ***P<0.001; n.s., not significant (P>0.05).

direction before and after collisions. In all conditions, over half of collisions showed values close to +1 (Fig. S3C), suggesting that worms did not change their direction after physical contact. In addition, we examined whether worms steer towards the odour after collisions (Fig. S3D,E). In all conditions, attraction values were uniformly distributed (Fig. S3D) and d*C*/d*t* before and after contacts showed a weak correlation (Fig. S3E, Table S1). The same results were obtained for *daf-22* mutants (Fig. S4). Taken together, although physical contact increases pirouette behaviour, this rarely affects chemotactic behaviours.

Crossing trails does not affect behaviours in chemotaxis

Animals, such as ants, that cross trails change their behaviours according to cues left by others (Sumpter, 2006; Steck, 2012). In our experiments, worms crossed trails many times (Fig. S5A). Thus, we examined whether crossing trails affected chemotaxis in *C. elegans* as in other species. For all conditions, the probability of pirouettes occurring within 10 s of worms crossing the trails was small (Fig. S5B). To examine whether crossing trails triggered pirouettes, we conducted a binominal test for each condition (Table S2; see Materials and Methods). The probability for each condition was not significantly different from the probability estimated from randomly selected time points. Therefore, crossing trails did not affect pirouette behaviour. Then, to investigate whether worms changed their direction after crossing trails, we compared the crossing angles against the direction of trails (Fig. S5C). The relationship between the direction before and after crossing showed a strong positive correlation (Fig. S5D, Table S1), suggesting that worms did not change their direction after crossing trails. The direction did not change depending on the worm's direction towards the odour source either (Fig. S5E,F). Therefore, crossing trails does not affect chemotactic behaviour in *C. elegans*.

DISCUSSION

Previous research has suggested that chemotactic behaviours can be represented as collective behaviours because of interactions between individual worms. To understand such interactions, we examined chemotaxis under three conditions: single, population and paired. Based on the trajectories to the odour source, worms in the population and paired conditions displayed different behaviours from those in the single condition. Further investigation showed that the threshold for temporal changes of odour concentration to initiate pirouettes became lower when worms interacted with each other. Finally, experiments conducted with the *daf-22* mutant suggested that this phenomenon resulted from interactions between individual animals via pheromones.

Thus far, analyses of behaviour involving chemotaxis have been performed on single worms. Here, we found differences in the behaviour of individual *C. elegans* relative to those in the collective conditions. From the experiments with the *daf-22* mutant, we revealed that the response to a temporal change of odour concentration driving pirouettes was altered by pheromones. This could explain why worms in the population and paired conditions moved away from each other (Fig. 1D). If pirouettes are driven by a

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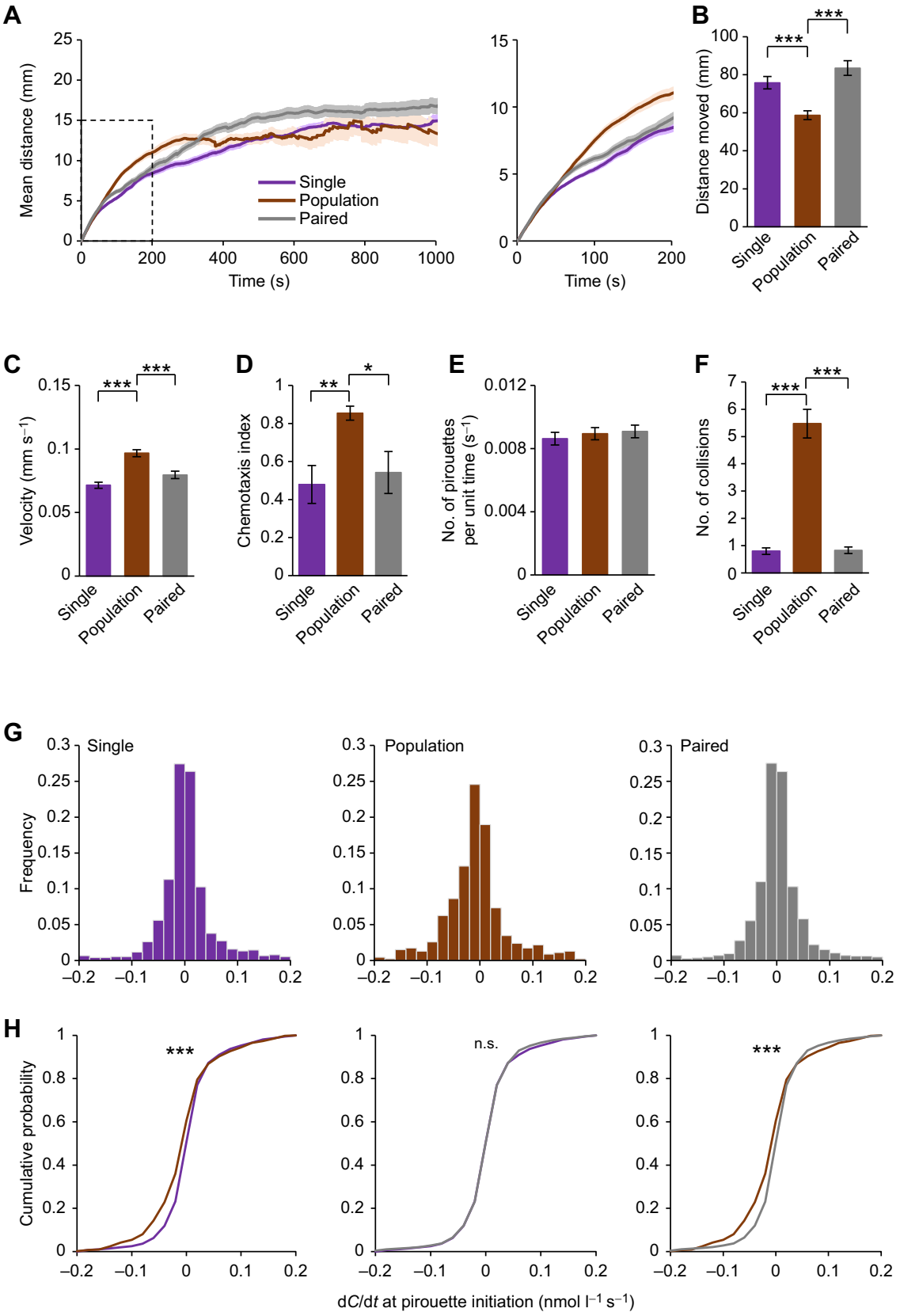

Fig. 3. See next page for legend.

Fig. 3. Collective behaviour of *daf-22* mutants in chemotaxis. (A) Mean distance between individual worms. Traces indicate the mean (with shading representing the s.e.m.) distance between neighbouring worms. The right graph is an enlargement of the area enclosed by the dashed line in the left graph. (B–F) Mean (±s.e.m.) distance moved (B), velocity (C), chemotaxis index (D), number of pirouettes per unit time (E) and number of collisions (F). (G) Histograms for commencement of pirouette behaviours in *daf-22* mutant worms under each condition (dC/dt). (H) Cumulative probability of the frequencies in G: left, single and population; middle, single and paired; right, population and paired [N=12, n=90, 92 and 94, respectively, for single (purple), population (brown) and paired (grey) conditions; N, n indicate the number of assays and animals, respectively]. B,E,F: Mann–Whitney U-test with Bonferroni correction; C,D: Student's t-test with Bonferroni correction; H: non-parametric two-sample Anderson–Darling test with Bonferroni correction; *P<0.05, **P<0.01, ***P<0.001; n.s., not significant (P>0.05).

large temporal change in odour concentration, worms would not initiate pirouette behaviours until they approached odour source. Therefore, the timing of the movements of worms in the population and paired conditions were delayed, leading to greater spread in their trajectories. This may prevent many worms from approaching attractants at the same time. The same role of pheromones in food exploration was reported (Greene et al., 2016a). In addition, other factors may regulate behaviours in populations. Although excessive physical contact changed chemotactic behaviours (Fig. 3), the physical contact itself did not seem to affect the phenomenon markedly (Figs S3 and S4).

The link between odour concentration and the performance of pirouette behaviour (Figs 2 and 3G,H) was perturbed because worms used pirouettes to avoid each other. We could not completely exclude disturbance from the analysis, but our results supported our conclusion. First, the characteristics of pirouettes in our experiments were the same as those in a previous study (Pierce-Shimomura et al., 1999) (Fig. S2). Pirouettes were triggered when dC/dt became negative, suggesting that pirouettes were triggered by a change in odour concentration. Second, if worms avoid each other, they have to be able to detect others by physical contact or pheromones. Thus, physical contact could be the main trigger for pirouettes, independent of the change in odour concentration. *daf-22* worms in the single and paired conditions showed fewer than two collisions with each other (Fig. 3F, Fig. S3A). This number is so small that it will have little influence. The high number of collisions for *daf-22* mutants in the population condition indicates that pirouettes were triggered independently of odour. The number of physical contacts in this condition was large (Fig. 3F), and the slope of the cumulative probability shifted to the left (Fig. 3H), indicating that the link between pirouettes and odour concentration was perturbed. Although we did not find out whether the pheromones themselves trigger pirouettes so that worms avoid each other, as mentioned above (Fig. S2), the effect is probably small. Taken together, the results (except for *daf-22* in the population condition) show that pheromones modulate the initiation of pirouettes depending on the change in the odour concentration, supporting our conclusion.

The regulation via pheromones changed the initiation rate of pirouettes in response to dC/dt. Pirouettes occurred when the time derivative of the odour concentration became negative (Pierce-Shimomura et al., 1999). In our results, the modulation was also particularly observed when dC/dt was negative (Fig. S2). As well as the pirouette strategy, the weathervane strategy is important for migration to the odour source. Our results did not show that this strategy altered between the single and collective conditions (Fig. S6). Although we concluded that the weathervane strategy was not affected in collective worms, our results could not exclude it as a cause of behaviour changes completely. If worms exhibited the weathervane strategy, the curving rate would increase against the vertical gradient along the direction of movement of the animals. However, the N2 strain did not display this trend (Fig. S6A). Thus, the influence of the weathervane strategy could not be observed in our experimental setup.

Interactions between two worms changed the initiation of pirouettes and induced collective behaviours. So far, research on collective behaviours has been performed on social animals. Our results show that the solitary *C. elegans* can be used as an experimental model along with other solitary species like *Drosophila* (Ramdya et al., 2015). These animals have advantages for genetic experimental approaches (Ramdya et al., 2017). In addition, tracking systems, which are important for studies on collective behaviours (Herbert-Read, 2016), have already been developed for *C. elegans* (Yemini et al., 2011; Husson et al., 2013); thus, the species can contribute to research on collective behaviour.

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Competing interests
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Author contributions
Conceptualization: T.Y., H.S., K.A., K.H., K.O.; Methodology: T.Y., H.S.; Software: T.Y., H.S., K.A.; Formal analysis: T.Y., H.S., K.A.; Investigation: T.Y.; Resources: H.S.; Writing - original draft: T.Y., H.S., K.A., K.H., K.O.; Writing - review & editing: T.Y., H.S., K.A., K.H., K.O.; Visualization: T.Y.; Supervision: K.H., K.O.; Funding acquisition: H.S.

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