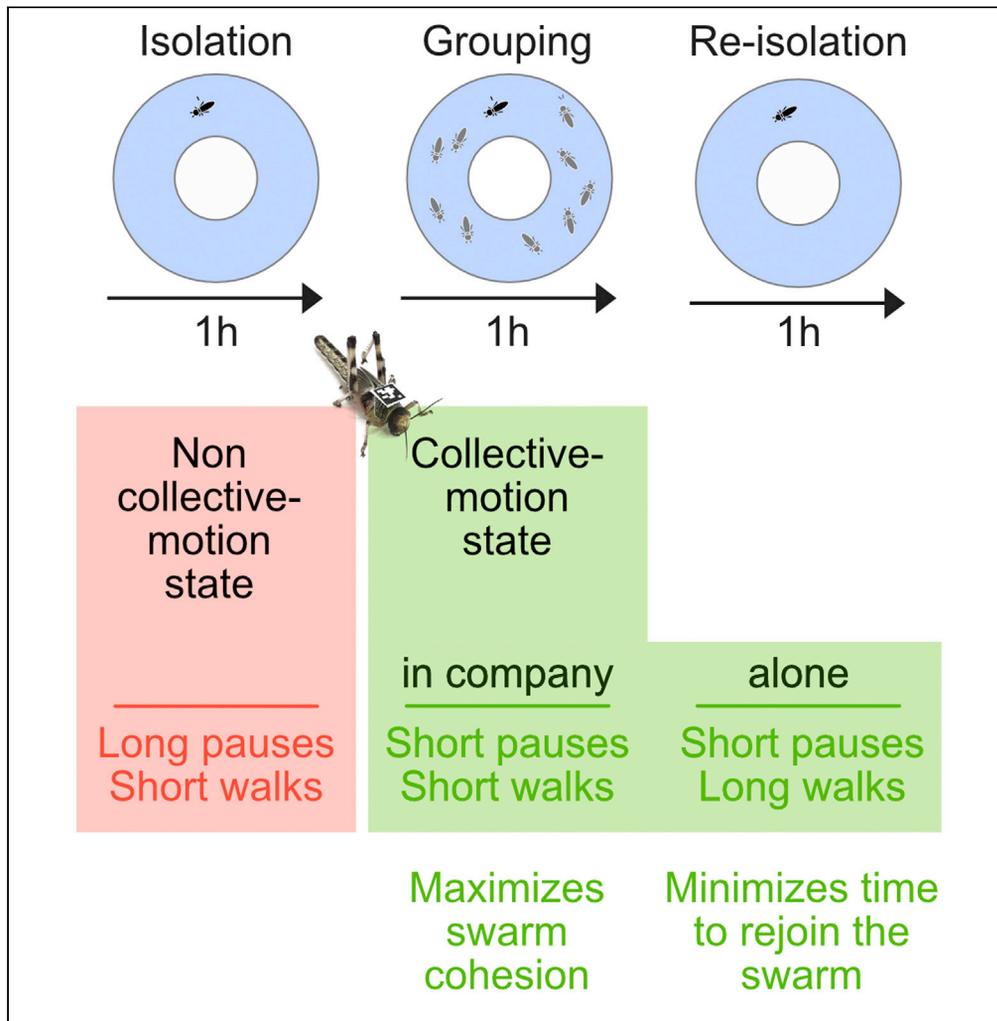**Article**

Collective motion as a distinct behavioral state of the individual

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Highlights

Locusts were monitored
before, during, and after
experiencing collective
motion

In each condition the
locusts showed distinct
walking kinematics

This indicates that the
locusts adopt collective-
motion-dependent
behavioral states

Simulations show that
these states may be
advantageous for the
swarm integrity

Article

Collective motion as a distinct behavioral state of the individual

Daniel Knebel,[1,2] Ciona Sha-ked,[1] Noa Agmon,[2] Gil Ariel,[3] and Amir Ayali[1,4,5,]*

SUMMARY

The collective motion of swarms depends on adaptations at the individual level. We explored these and their effects on swarm formation and maintenance in locusts. The walking kinematics of individual insects were monitored under laboratory settings, before, as well as during collective motion in a group, and again after separation from the group. It was found that taking part in collective motion induced in the individual unique behavioral kinematics, suggesting the existence of a distinct behavioral mode that we term a "collective-motion-state." This state, characterized by behavioral adaptation to the social context, is long lasting, not induced by crowding per se, but only by experiencing collective motion. Utilizing computational models, we show that this adaptability increases the robustness of the swarm. Overall, our findings suggest that collective motion is not only an emergent property of the group but also depends on a behavioral mode, rooted in endogenous mechanisms of the individual.

INTRODUCTION

The ability to form groups that move collectively is a key behavioral feature of many species (Sumpter, 2006; Ward and Webster, 2016), assumed to increase the survival of both individuals and groups (Be'er and Ariel, 2019; Yang and Schmickl, 2019). Collectively moving organisms, however, differ in the levels of peer-to-peer interactions, ranging from minimal cooperation to complex social behaviors (Attanasi et al., 2014; Cavagna et al., 2010). Furthermore, endogenous differences among individuals, heterogenic environments, and variability in the interactions between the individual and its direct environment are all sources of variance that may affect the coordinated behavior of the collective. Accordingly, it is not clear how synchronized collective motion constitutes such a robust phenomenon, maintaining its form across various group sizes and densities, and under heterogeneous and unpredictable environmental conditions.

One of the most interesting, albeit disastrous, examples of collective motion is that of the marching of locusts. These insects swarm in groups of millions, migrating in mass across large distances, devastating vegetation, and agriculture (Ayali, 2019; Cullen et al., 2017; Zhang et al., 2019). In the context of social interactions, locust swarming is characterized by a minimal level of cooperation between individuals: collectivity, which is based on local interactions, is mostly manifested in alignment among neighboring individuals and in maintaining the overall movement in the same general direction (e.g., Ariel et al., 2014a; Bazazi et al., 2008). Nonetheless, the locust swarming phenomenon is extremely robust, with huge swarms demonstrating moderate to high collectivity on huge scales (up to 6–7 orders of magnitudes), in terms of both the number of animals and their spatiotemporal distribution (Ellis and Ashall, 1957; Uvarov, 1977; see further references in Ariel and Ayali, 2015). Thus, locusts exhibit a considerable disparity between little local cooperation and large-scale collectivity.

What is the key to this ability of locust swarms to maintain their integrity? Here, we show by a series of carefully controlled behavioral experiments that collective movement induces an internal switch in the individual gregarious locust, activating a behavioral mode we refer to as a "collective-motion-state." In this state, the kinematic behavior of individuals notably differs from that during a non-collective-motion-state. It is important to emphasize that both the "collective-motion-state" and the "non-collective-motion-state" are internal states of swarming-gregarious locusts. We are not referring to the well-known solitarious-gregarious phase transition in locusts (Ayali, 2019; Cullen et al., 2017).

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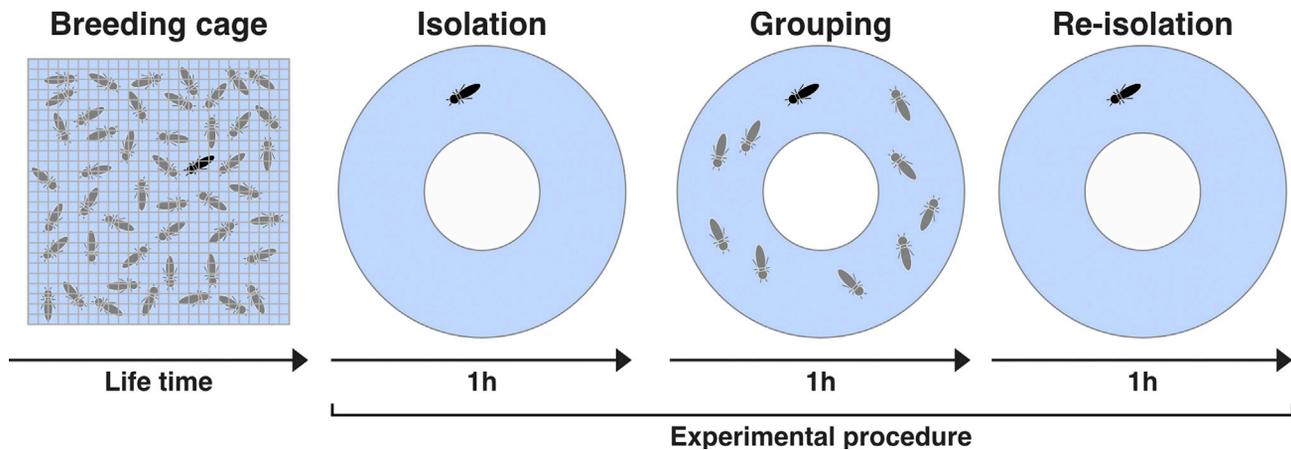https://doi.org/10.1016/j.isci.2021.102299

Figure 1. A schematic flow of the experimental procedure

Locusts were reared in high-density conditions. The Experiments comprised the following consecutive stages: (1) isolation for 1 h in the arena, (2) grouping for 1 h, and (3) re-isolation for 1 h.

How, then, does the collective-motion-state affect the formation and robustness of the swarm? Interestingly, the switch into this state seems to occur rapidly, and in response to coordinated walking. In particular, our experiments indicate that aggregation alone is not sufficient. Switching out of the collective-motion-state occurs over a longer timescale—significantly longer than the typical timescale of normal fluctuations around the swarm typical dynamics. Hence, stochastic fluctuations, typical to swarming behavior (Algar et al., 2019; Ariel and Ayali, 2015; Escaff et al., 2018), are "smoothed-out," leading to highly robust dynamics of the swarm collective behavior, which is in turn beneficial for the swarm integrity.

Using a simplified computer model, we simulated the swarming properties of locust-like agents with different kinematic parameters, representing the different behavioral states. The results support the functional advantages of the collective-motion-state, allowing us to conclude that the collective-motion-state provides an individual-based mechanism that increases the stability of swarms in the presence of fluctuations, preventing the swarm from collapsing.

RESULTS

The main objective of this report was to examine the behavior of individual animals upon joining and mostly leaving a group of conspecifics. Here we studied gregarious locusts, reared in dense populations, one developmental stage before becoming adults and developing functional wings (i.e., fifth-instar larva). The experiments comprised three consecutive stages, representing different conditions (Figure 1 and Video S1): (1) Isolation stage: a single animal was taken from its highly dense rearing cage, tagged with a barcode, and introduced alone into a ring-shaped arena (outer and inner diameters: 60 and 30 cm, respectively). (2) Grouping stage: nine other individually tagged animals were added to the arena. (3) Re-isolation stage: the nine added animals were removed from the arena, leaving the original animal alone. The duration of each stage was 1 h, which was enough for the locusts to exhibit their walking kinematics, yet did not cause behavioral changes due to exhaustion, hunger, etc. The trajectories of the animals were fully reconstructed using a barcode tracking system. The middle 40 min of each stage were analyzed, as detailed in the transparent methods section. A range of kinematic statistics was collected to classify and compare the locusts' behavior in the different stages.

Swarm formation—validation of collective motion

To verify that our grouping conditions were indeed inducing collective motion (swarming), we calculated the synchronization in movement of the grouped animals using the order parameter (see transparent methods for definition), which is a fundamental estimator for the typical marching behavior of locusts (e.g., Knebel et al., 2019). The median order parameter in the grouping stage was found to be significantly higher than that obtained for computationally randomized groups (as presented in our previous report, Knebel et al., 2019; medians: 0.632 and 0.239, respectively; Wilcoxon rank-sum test: $p < 0.001$).
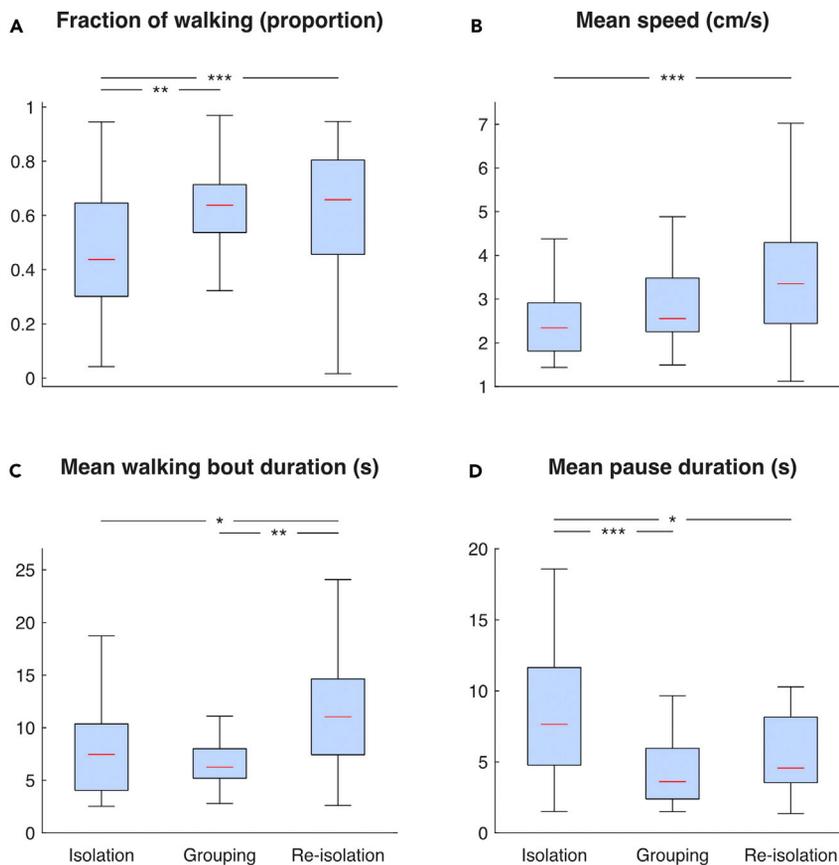
Figure 2. Kinematic changes throughout the three experimental conditions

(A–D) (A) The fraction of walking, (B) the averaged walking speed, (C) the average duration of walking bouts, and (D) the average duration of pauses of the traced animals in the isolation, grouping, and re-isolation stages. Red lines denote the median. Boxes show the interquartile range (25th to 75th percentiles). Whiskers are the max and min data points (excluding points that are more than 1.5 times the interquartile range away from the bottom or top of the box). *p < 0.05, **p < 0.01, ***p < 0.001.

Consequently, we conclude that the groups in our experimental setup indeed demonstrated swarming and collective motion.

Kinematic differences among the isolation, grouping, and re-isolation stages

Locusts walk in an intermittent motion pattern (Ariel et al., 2014a; Bazai et al., 2012), i.e., movement occurs in sequences of alternating walking bouts and pauses. To characterize individual locust kinematics, we measured four parameters: (1) the fraction of time an animal spends walking, (2) the average speed while walking, (3) the average walking bout duration, and (4) the average pause duration. Comparing these values across the three experimental stages, we found several statistically significant differences (Figure 2). In the following, p values correspond to a Friedman test followed by a multiple comparison test using the Bonferroni method.

We found that when comparing between isolation and grouping, the fraction of time spent walking and the pause durations differed significantly ($p < 0.01$ and $p < 0.001$, respectively), showing a larger fraction of time walking and shorter pauses while grouping. These findings are in accordance with previous reports (Knebel et al., 2019), and are consistent with the known propensity of locusts to walk more and rest for shorter times while in a swarm (Ariel et al., 2014a; Bazai et al, 2008, 2012; Knebel et al., 2019). However, our experiments also revealed a new effect of swarming. Comparing the isolation and re-isolation stages, we found that all the studied parameters differed significantly. Specifically, the fraction of time walking, speed, and walking bout duration were all higher in the re-isolation stage, whereas the pause duration decreased ($p < 0.001$, $p < 0.001$, $p < 0.05$, and $p < 0.05$, respectively). Interpreting these parameters together, while also taking
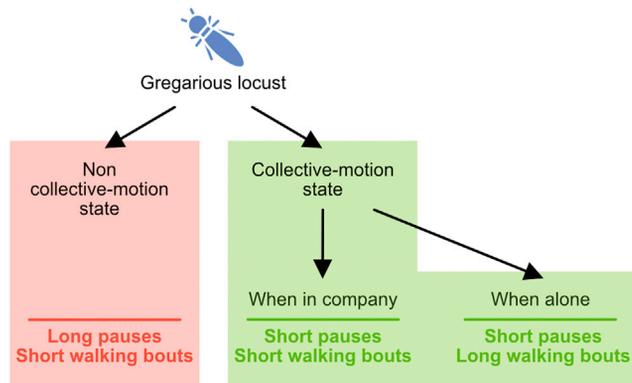
Figure 3. A schematic representation of the behavioral states of the locusts

into account the low propensity of locusts to turn while walking (or to make a U-turn upon starting to walk; Ariel et al., 2014a), the overall area explored by the locusts was much larger during the second isolation state. Furthermore, comparing the grouping and re-isolation conditions revealed that the walking bout duration increased significantly following re-isolation (p < 0.01). The data for all other comparison combinations were found not to differ significantly. The exact data points and trends can be found in Figure S1.

The increase in activity following re-isolation is surprising and suggests that the marching behavior of locusts is not dictated by instantaneous or immediate interactions among individuals per se. Rather, our findings indicate that the interactions with other marching locusts induce a switch to a new internal behavioral state, which outlasts the presence of the swarm. In accordance with our results, we term this internal state the "collective-motion-state." The different behavioral states are schematically presented in Figure 3.

To verify that the observed behavioral changes indeed represent a transient state, rather than a permanent behavioral modulation, a simple control experiment was performed. In six of the experiments, following the re-isolation stage, locusts were returned to their rearing cage (in high crowding conditions without collective motion) and tested the next day again alone in the arena (isolation condition). We found no significant behavioral difference between this latter isolation and the first isolation stage of the previous day. Hence the collective-motion state is transient. A second series of control experiments (n = 6) was performed to exclude potential time effects on the locusts' behavior due to the duration of the experiments. To this end, locusts were tested in isolation for three consecutive hours. The above-described kinematic analysis procedure was then performed separately on three 40-min segments of the 3-h tests, and no significant differences were found.

Consistency of individual behavioral tendencies

Despite the observed major differences in behavioral kinematics among the three experimental stages, we were also interested to know whether there are any correlations between the changing parameters in the three experimental conditions: isolation, grouping, and re-isolation. This would indicate that although individuals change their behavior throughout the experimental stages (Figure 2), they maintain the relative position when compared with others, and thus show some consistent individual tendencies. We found that individual behavioral tendencies generally persisted. The fraction of walking, speed, and pause durations all showed high within-individual correlation across the three stages. The walking bout durations, however, were significantly correlated only between the isolation and grouped stages, but not between re-isolation and the other stages (see Table 1 for numerical details). This suggests that whereas the fraction of walking, speed, and pause durations are highly dependent on the animal tested itself, the bout duration during re-isolation cannot be predicted by the previous stages, and is therefore influenced by the social context rather than by the animal's unique properties.

Modeling and simulations

The above-described experiments demonstrated that individual locusts introduced into a collectively moving swarm undergo a switch into a distinct internal sociobehavioral state. However, whether this change confers a benefit on the swarm formation and maintenance, and if so, of what kind, remained unanswered.

Table 1. Correlation values between kinematic parameters throughout the three experimental conditions

	p value	rho value
Fraction of walking		
Isolation-grouping	<0.001	0.706
Isolation-re-isolation	0.001	0.679
Grouping-re-isolation	0.002	0.638
Speed		
Isolation-grouping	0.001	0.679
Isolation-re-isolation	0.010	0.561
Grouping-re-isolation	0.002	0.636
Walking bout duration		
Isolation-grouping	0.002	0.628
Isolation-re-isolation	0.148	0.391
Grouping-re-isolation	0.354	0.314
Pause duration		
Isolation-grouping	0.007	0.580
Isolation-re-isolation	0.003	0.612
Grouping-re-isolation	<0.001	0.708

The fraction of walking, the averaged walking speed, the average duration of walking bouts, and the average duration of pauses were tested for correlation across the three experimental conditions: (1) isolation, (2) grouping, and (3) re-isolation. The underlined values mark significant correlations.

To explore this aspect of the collective-motion-state, we developed a model that simulates locust swarms, in which individual kinematics could be manipulated.

To simulate swarms, we used a simplified agent-based model in a square domain with periodic boundaries. Agents were designed as rectangles with a circular receptive field around their center. The agents' kinematics was programmed to resemble that of locusts, i.e., to move in an intermittent motion (pause-and-go) pattern: at every step of the simulation, each agent made an individual decision whether to walk or stop, based on its current state (walking or stopping) with predefined probabilities (p_W and p_S, respectively; Figure S2). While moving, the speed was constant. The individual direction of movement was allowed to change only when an agent changes its state from stopping to walking (Ariel et al., 2014a). An agent's new direction was a weighted sum of its own direction (inertia), the direction of other agents in its visual field, with a short memory (see Rimer and Ariel, 2017 for the importance of memory in pause-and-go simulations), and noise. These were set to generate an order value approximately similar to that obtained in our experiments. See transparent methods for details and Table S1 for parameter values.

The spatial and temporal scales of the model were set as follows: 1 cm was considered as a distance of 0.1 in the simulated arena, and 1 s corresponded to 1 simulation step. Thus, we fixed the model dimensions to correspond with real locusts' size and movement parameters. The size of agents (0.1 × 0.4) maintains the proportions of fifth-instar larva locusts. Additionally, the visual range of 1 radius, 10 times larger than the agent's rectangle width, represents the proportional visual field of locusts (range just under 10 cm; Ariel et al., 2014a). The speed was set to 0.25, corresponding to the typical swarming speed shown in Figure 2B. Finally, the size of the arena was set to be 7 × 7, with 12 agents within. This generated a slightly lower density than in the real experiments, but better mimicked the limited visual field the locusts experienced in our ring-shaped experimental arena.

We evaluated the effect of the agent's walking bout and pause durations, controlled by p_W and p_S, on four statistics that characterize collective motion in the swarm:

1. The order parameter (the size of the average direction vector).

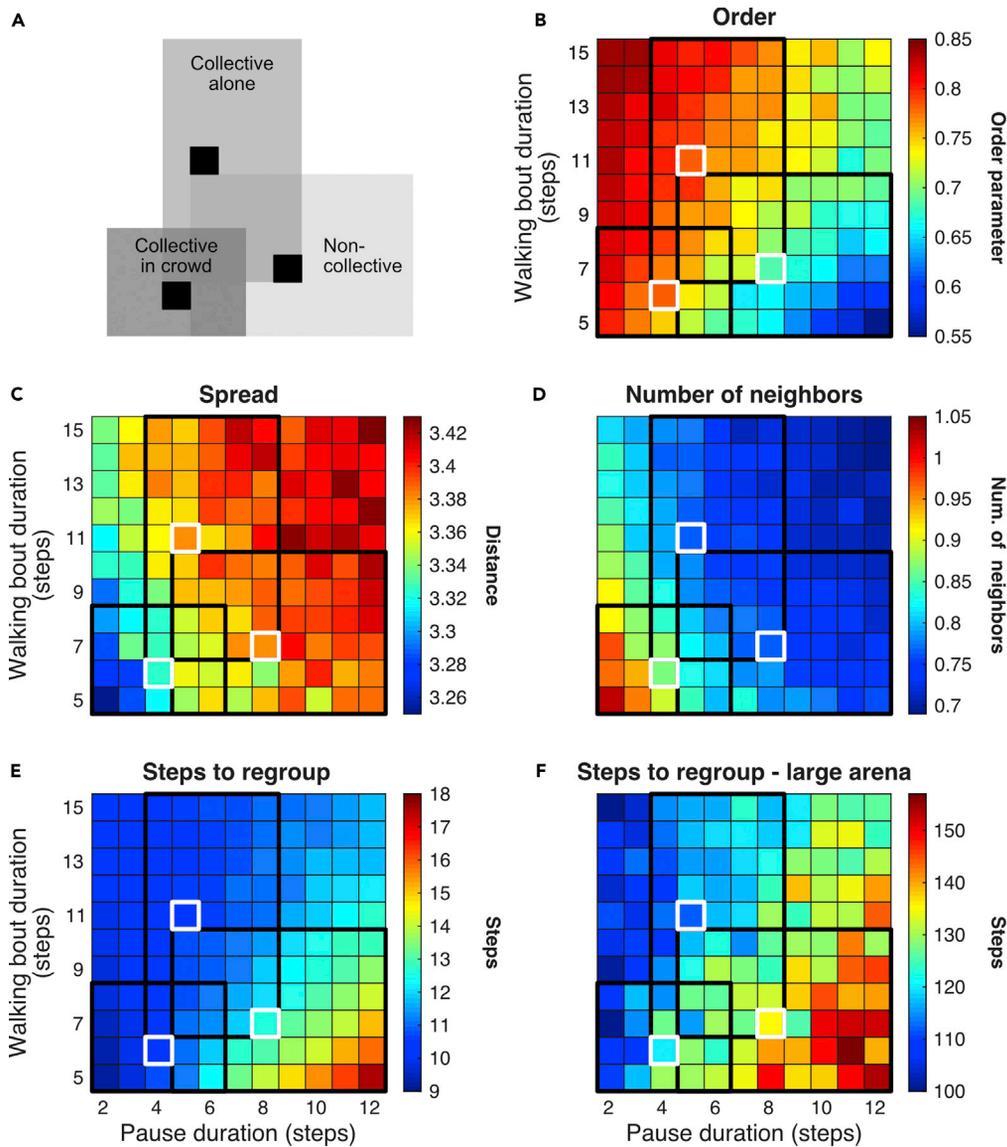2. Spread (the average distance between all pairs).

Figure 4. The influence of different walking bout and pause durations on the collectivity parameters in simulated swarms

(A) The areas representing the behavioral states in the next traces, where the black and white frames indicate the interquartile ranges and medians of each condition, respectively, obtained from Figures 2C and 2D.

(B–E) (B) The order parameter, (C) spread, (D) the average number of agents within each agent's visual field, and (E) the average number of steps to regroup, for different walking bout (rows) and pause (columns) average durations. The arena size is 7 × 7 with periodic boundaries.

(F) The average number of steps to regroup in a large 75 × 75 arena, with the same number of agents. In (B–E) each colored box represents the median of 40.

3. The number of neighbors (within the field of view, denoted NN).

4. Regrouping time (the average number of steps it takes an agent that has lost all other agents in its receptive field to re-obtain at least one neighbor).

Statistics were averaged over all agents and simulation steps

Figure 4 shows the median value over 40 independent repetitions for each parameter set. We found that the order parameter increases with the walking bout duration but decreases with pause durations

(Figure 4B). The spread, on the other hand, is increasing both with walking and pausing durations (Figure 4C). The NN statistics (Figure 3D) were in accord with the spread (small spread implies many NN). Its values were similar to that obtained in the real experimental arena (∼1–1.5), which was slightly denser, as explained earlier. The regrouping time (Figure 4E) showed a more complex dependency on the duration of walk and pause durations: it was shortest when both the parameters were low and longest when the walking bout was low but the pause duration was high. Yet, for mid values of pause duration, high walking bouts durations induced a reduction in the number of steps to regroup. Worth noting is the fact that because agents could only change direction when starting to move (similar to the locusts; Ariel et al., 2014a), the length of walking segments between turns increases with walking durations.

To relate the simulation and experimental results, the three collective motion states—isolation, grouping and re-isolation—were indicated in Figure 4 by the tiles corresponding to the interquartile range of the empirical data presented in Figures 2C and 2D. Additionally, we also marked the corresponding median data point. As can be seen, in all the parameters calculated, the individual behavior that reflects the collective state in a crowd improves the coherency and rigidity of the swarm. This important observation suggests a possible benefit for increasing the walking bout duration when a locust is in a collective state, but finds itself alone.

To further explore the advantages of a collective motion step in re-isolation scenarios, we increased the size of the arena to 75 × 75, with the same number of agents, thereby reducing the density considerably, and calculated the steps to regroup parameter (Figure 3E). We found that for low density, where practically no collective behavior is present (see Figure S3), longer walking bouts reduce the time to regroup. Therefore, it is beneficial for an agent that finds itself alone due to a sparse distribution of the swarm to increase its walking bouts duration (even at the cost of decreasing other parameters of collectivity), and thus shorten the time until it reunites with other locusts.

DISCUSSION

Our findings reported here suggest that, in locusts, the sensorimotor act of collective motion is accompanied by an internal state of the individual locust—a collective-motion-state, which is manifested in specific behavioral kinematics. This state is induced by the experience of synchronous, collective marching. In turn, it has an important role in maintaining the integrity and consistency of the swarm. Next, we discuss several key aspects and implications of this finding.

It should be stressed once again that the current study focused on gregarious, crowded-reared locusts only. The described behavioral states should not be confused, therefore, with the well-known and much researched locust density-dependent phase polyphenism (Ayali, 2019; Cullen et al., 2017). Collective motion is limited to the gregarious, swarming, and migrating phase. Accordingly, all our experimental animals were taken from our gregarious (crowded-reared) breeding colony, maintained in crowded conditions for many consecutive generations. In their breeding cages, mostly due to the physical constraints and abundance of food, despite experiencing high density, the locusts very rarely, if at all, demonstrate collective motion. Thus, they adopted the collective-motion-state only upon experiencing, and taking part in, collective marching within the experimental arena.

In a recent study (Knebel et al., 2019), we have introduced a comparison between the walking behavior kinematics of individual gregarious locusts in different social (density) contexts. Our reported findings are reconfirmed and further elucidated here by the results of the initial isolation and grouping stages in our experiments. The novel idea posited here is that these differences represent not only the spatially and temporally immediate social environment and the instantaneous local interactions among locusts but also are dictated by the effects of an internal state induced by the general experience of collective motion. A fundamental aspect of the concept of the collective-motion-state arises from our findings related to its persistent effect in time: upon re-isolation, the individual locust adopted behavioral kinematics that critically differed from that in the first experimental stage (initial isolation). We also showed that, as expected, the collective-motion-state is transient. If the locust does not experience collective motion for some time, and is then isolated once more, it loses the unique walking-related kinematics it previously adopted in response to the collective motion, i.e., the internal collective-motion-state. The dynamics of this decay were not explored, but are likely to be affected by many external factors, such as the availability of food and the day-night cycle.

The individual locusts in our experiments retained the variability demonstrated in our previous report (Knebel et al., 2019), while demonstrating a second layer of variability or plasticity upon experiencing collective motion, when entering the collective-motion-state. Considerable research has been devoted in recent years to understanding the effect of variability among individuals on the group's collective behavior, both experimentally—ranging from bacteria to primates (Benisty et al., 2015; Brown and Irving, 2014; Crall et al., 2016; Dyer et al., 2009; Farine et al., 2017; Fürtbauer and Fry, 2018; Herbert-Read et al., 2013; Jolles et al., 2018; Planas-Sitjà et al., 2015)—and theoretically (Aplin et al., 2014; Ariel et al., 2014b; Calovi et al., 2015; Copenhagen et al., 2016; Guisandez et al., 2017; Jolles et al., 2017; Menzel, 2012; Mishra et al., 2012; see Mar Delgado et al., 2018; Modlmeier et al., 2015; Webster and Ward, 2011 for recent reviews). The interactions between variability in specific aspects of the individuals' behavior and group-level processes were found to be complex and, moreover, bidirectional (e.g., Knebel et al., 2019). Variability among individual animals was found to have important consequences for the collective behavior of the group (e.g., O'shea-Wheller et al., 2017; Szorkovszky et al., 2018).

However, beyond the variability among the individuals composing a group, variability is also expected in the behavior of the individual animal over time, as it experiences changes in environmental and social conditions. The swarm (or flock, shoal, herd, etc.) is a heterogeneous entity, moving in a heterogeneous environment. The individual is bound on occasion to find itself in different locations within the swarm (e.g., leading edge, at the outskirts, trailing), and it may also find itself separated from the group by natural obstacles (vegetation, rocks, and boulders). It is essential for the robustness and consistency of the swarm that throughout these changing conditions the behavior of the individual will adapt accordingly, such as to be appropriate for the changing context. For example, if temporarily separated from the core of the swarm, a locust's walking kinematics should change to support rapid reunion with the group, as reported in both our experimental and simulation findings (e.g., increased fraction of walking and duration of walking bouts). If previously naive to collective motion, that individual's kinematics would, however, be disadvantageous, or even hinder the formation of a swarm.

In Bazazi et al., 2012, the authors suggest that behavioral variability can be explained by the existence of two internal states. Studying single locusts in isolation for 8 consecutive hours, they have observed changes in behavioral kinematics that were suggested to result from "internal state behavioral modulation." The observed variations, however, were merely attributed to changes in "starvation/satiation state," i.e., as the locust becomes starved, it changes its walking behavior, searching more vigorously for food. Moreover, they conclude that animals continually switch between the two states on a scale of minutes. The collective-motion-state reported here is, of course, a very different type of internal behavioral state, which is strongly involved with the locust past and current social environment. It may be viewed as a form, or a manifestation of a social carryover effect (Niemelä and Santostefano, 2015), where a social environment experienced by a focal individual affects aspects of its locomotion behavior at a later, non-social context. As noted, however, the change in behavioral state described here is induced by collective marching, i.e., a particular mode of social interaction, rather than by aggregation or being around other conspecifics per se. Moreover, as our simulations show, the enhanced marching displayed in the re-isolation stage is advantageous for maintaining collective swarming—it is still much related to the social context rather than carried over to a non-social one.

The reported collective-motion-state is also in accord with the overall daily behavioral changes of marching locust swarms. The swarm will spend the night (as well as times of low temperature or other unfavorable climatic conditions) roosting among the vegetation. Upon suitable conditions, after a period of feeding, the locusts will initiate marching—highly synchronized, collective motion. Frequently, when temperature becomes too high around noon, or when dusk arrives, the swarm will again switch to feeding and roosting. These daily patterns call for corresponding changes in the internal behavioral states of the individual locusts and mostly a dedicated collective-motion-state.

In the current work we are cautious in discussing the underlying mechanisms of the behavioral states reported. Although this is beyond the scope of this study, it is clear that these behavioral states represent physiological states. With some confidence, we can speculate about the nature or the physiological mechanisms involved in the demonstrated behavioral states. Behavioral plasticity in locust behavior has been attributed to various second messengers or neuromodulators, or to the balance among them. Most notable are the biogenic amines (e.g., serotonin, a prominent bio-amine, was recently reported to inhibit

walking behavior in *Drosophila*; Howard et al., 2019). Hence, it may well be that the (spatial and temporal) immediate social environment affects biogenic amine levels, and these in turn modulate the walking-related behavioral kinematics manifested in the different behavioral states.

Another candidate that may be involved in the collective-motion-state is the locust adipokinetic hormone (AKH). AKH is a metabolic neuropeptide principally known for its mobilization of energy substrates, notably lipid and trehalose, during energy-requiring activities such as flight and locomotion, and also during stress (e.g., Perić-Mataruga et al., 2006). It is well accepted that the metabolic state affects the level of general activity of an organism, and AKHs are reported to stimulate locomotor activity, either directly by way of their activity within the central nervous system (e.g., Wicher, 2007) or via octopamine—a biogenic amine with ample behavioral effects (Verlinden et al., 2010; Yang et al., 2015).

Furthermore, as noted, we have demonstrated here an extended effect of the experience of collective motion. Hence, learning and memory-related mechanisms would also seem to be involved. Again, previous work may suggest some candidate molecules and pathways, including cGMP-dependent protein kinase (PKG) and protein kinase A (PKA) (Geva et al., 2010; Lucas et al., 2010; Ott et al., 2012).

Last, as noted, solitarious phase locusts lack the capacity to demonstrate collective motion, and thus also the collective-motion-state. Accordingly, they differ from gregarious locusts in all the above-mentioned physiological pathways (bioamines: e.g., Alessi et al., 2014; Cullen et al., 2017; Ma et al., 2015; AKH: Ayali and Pener, 1992; Pener et al., 1997; PKG: Lucas et al., 2010; PKA: Ott et al., 2012). An in-depth investigation of the development of gregarious-like states in solitary locusts should prove to be very enlightening.

A central question is whether a collective (herd, flock, or swarm) is merely a sum of its parts, or a new entity. Most related studies have perceived collectivity as a self-emergent phenomenon, suggesting that new dynamics and behavior are the result of intricate, multi-body, typically non-linear interactions (e.g., Cucker and Smale, 2007; Vicsek and Zafeiris, 2012). One hidden assumption underlying this perception is that individuals remain inherently unchanged when isolated or in a crowd. Even studies of heterogeneous swarms, in which conspecifics may differ from each other, still assume consistency in the properties of the individual over time. This is essentially a physical point of view, in the sense that agents/individuals possess certain properties that determine their behavior across a range of situations. Thus, the collective motion is an emergent property that builds up in particular contexts, such as a sufficiently high local density of animals. This point of view allows, among others, extrapolation from experiments with one, two, or a few animals to large swarms (e.g., Calovi et al., 2015).

Our findings reported here suggest a fundamentally different point of view. We perceive the sensorimotor act of collective motion as accompanied by an internal state—a collective-motion-state that is manifested in specific behavioral kinematics. This state is induced by the experience of synchronous, collective motion. Most importantly, it is *not* induced by spatial aggregation alone. Collectivity, therefore, is not just self-emerging. Rather, the collective-motion-state has an important role in maintaining the integrity and consistency of the swarm. The robustness of the swarm is also a major challenge and requirement in swarming robotics, making the current novel insights applicable and even important also to this emerging field.

In the case of locusts, our far-from-complete understanding of the swarming phenomenon is also proving crucial for human well-being and survival, as evident from the current devastating locust situation in large parts of Africa and Asia (FAO, 2020). Much scientific attention has been dedicated to the perception, decision-making, and individual kinematics of locusts in a swarm. These efforts have led to various models that attempt to explain the collective behavior on the basis of local interactions among the individual locusts (see Ariel and Ayali, 2015 for review). The current study is, to the best of our knowledge, the first to include the internal state of the individual locust as an important factor in dictating its behavior, and in turn affecting the maintenance and the properties of the swarm.

Limitations of the study

The study presented here outlines a post-swarming behavioral state of individuals. Clearly, as noted, this state is induced by neurochemical changes such as secretion of neuromodulators and/or hormones. Yet, it was beyond this research to pinpoint the exact neuronal mechanisms involved. Furthermore, the presented model is simplified and ignores various aspects of locust swarming that might be critical. However, the

simplicity is also a virtue of the model, which can be easily generalized to other systems. In addition, although we show that the collective-motion-state is transient, we did not explore its temporal materialization and decline.

Data and code availability

The data will be made available upon request.

METHODS

All methods can be found in the accompanying transparent methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.102299.

AUTHOR CONTRIBUTION

D.K., G.A., and A.A. designed the study. D.K. and C.S.-k. performed the experiments. D.K. and C.S.-k. analyzed the data. D.K. N.A., and G.A constructed the model. D.K. wrote the code, performed simulations, and analyzed the data. D.K., G.A., and A.A. wrote the manuscript. All authors reviewed and approved the paper.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

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Supplemental information

Collective motion as a distinct

behavioral state of the individual

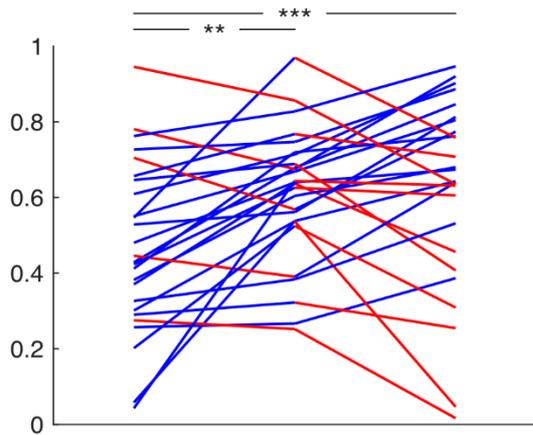Daniel Knebel, Ciona Sha-ked, Noa Agmon, Gil Ariel, and Amir Ayali
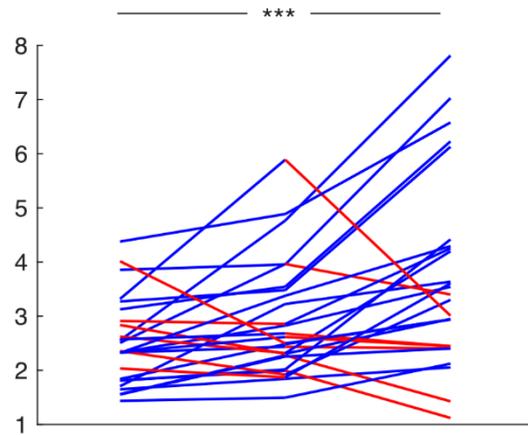
Supplemental information

Figures and tables

|	Variable	Value
Simulation parameters	Num. of agents (N)	**12**
	Num. of steps	**1000**
	Arena size (periodic boundaries; L)	**7 x 7 / 75 x 75**
Agents dimensions	Size of agents ($a1$ x $a2$)	**0.1 x 0.4**
	Visual range radius (r)	**1**
Movement parameters	Probability to stop (p_s)	**0.5 - 0.917**
	Probability to walk (p_w)	**0.8 - 0.933**
	Speed (v)	**0.25**
Direction parameters	Inertia weight (q_1)	**0.2**
	Current surrounding weight (q_2)	**0.2**
	Memory surrounding weight (q_3)	**0.3**
	Memory length (of surrounding memory)	**5**
	Random vector weight (q_4)	**0.3**

Table S1. Model parameters.
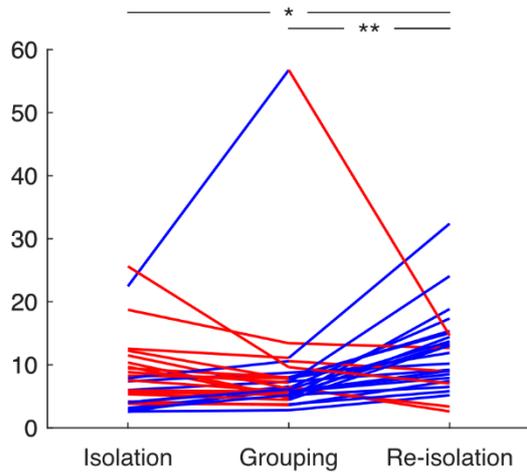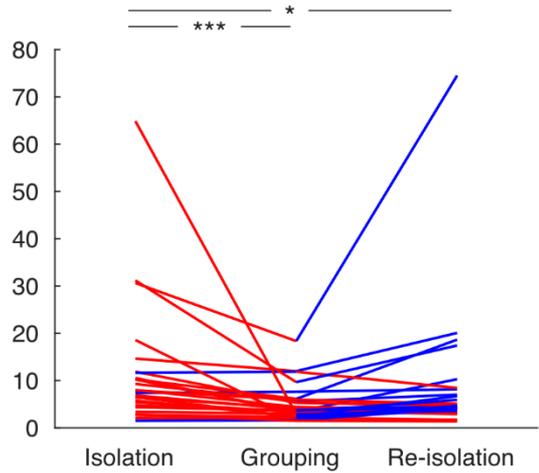
Fig. S1. Kinematic changes throughout the three experimental conditions – exact data points and trends. (A) the fraction of walking, (B) the averaged walking speed, (C) the average duration of walking bouts, and (D) the average duration of pauses, of the traced animals in the isolation, grouping, and re-isolation stages. Blue lines represent increase and red lines decrease between consequent experimental conditions of individual animals (n=26). *$p<0.05$, **$p<0.01$, ***$p<0.001$.
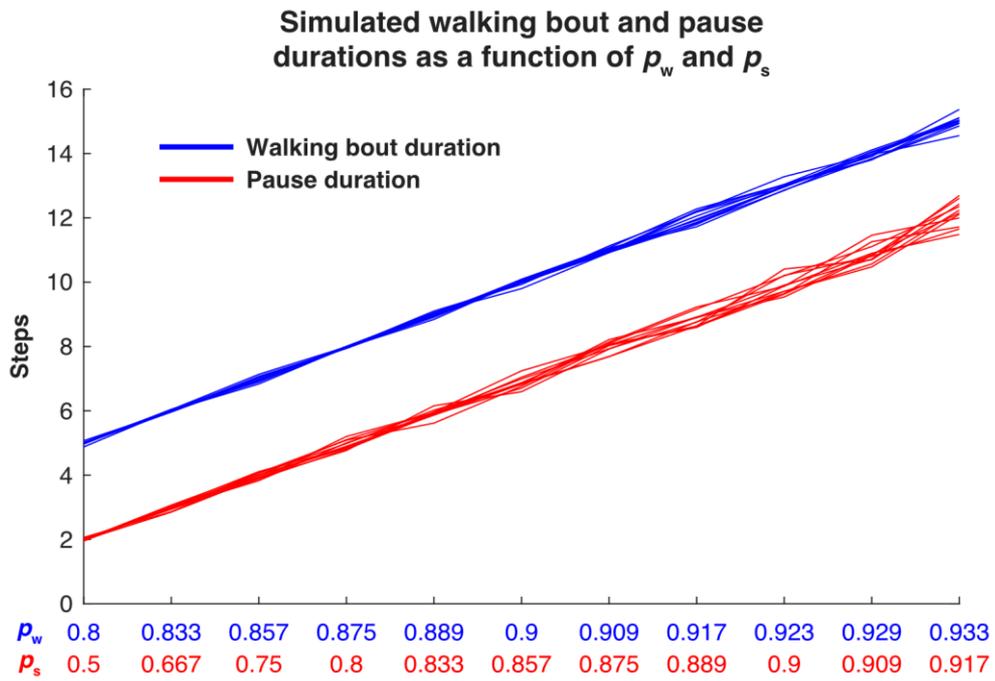
Figure S2. Walking and pause durations for different p_w and p_s values. The median bout and pause durations presented in Fig. 4, arranged by their corresponding p_w and p_s (blue and red, respectively).
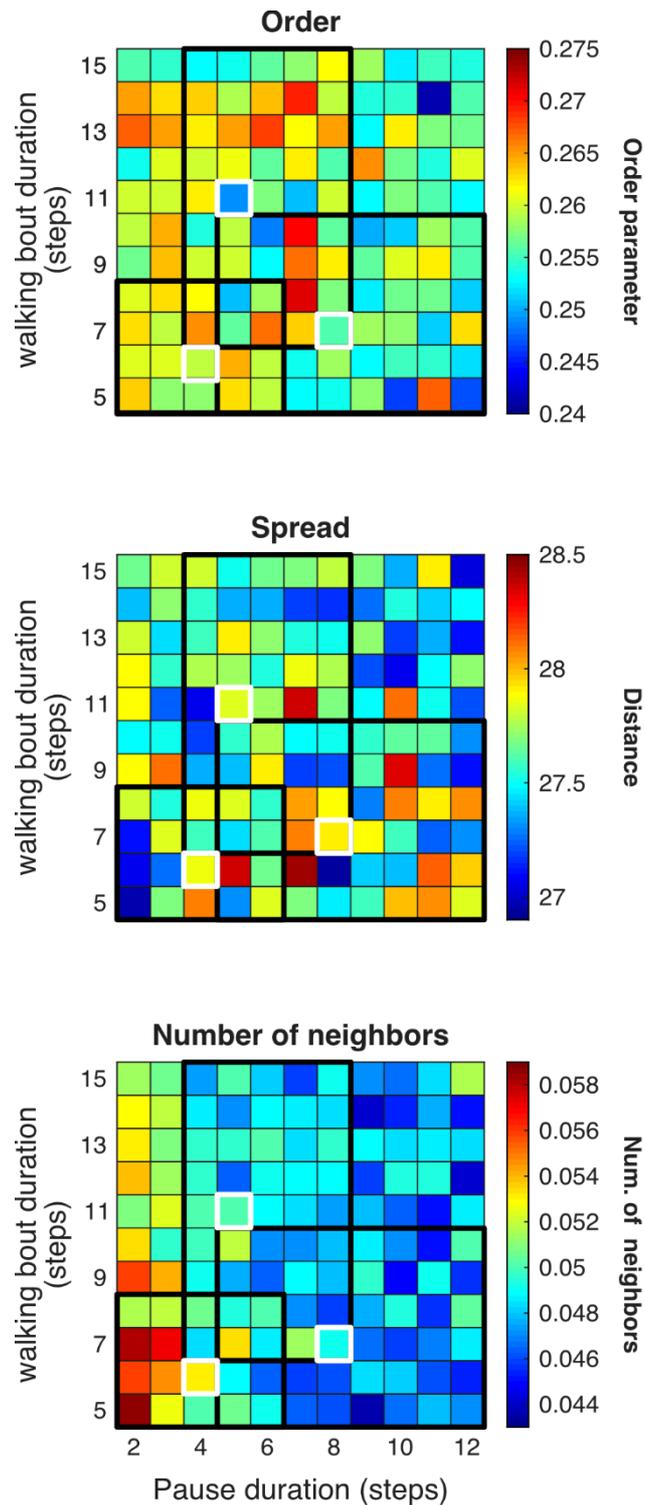
Figure S3. The influence of different walking bouts and pauses duration on the collectivity parameters of simulated swarms in an arena of 75x75. The order parameter, spread and the average number of agents within each agent's visual field for different walking bouts (rows) and pauses (columns) durations. Notice that the low numbers and minor changes between the boxes indicate that there was minimal to no collective behaviour in the 75x75 sized arena. Each box represents the median of 100 simulations.

Transparent Methods

Animals

The empirical experiments were conducted on gregarious desert locusts, *Schistocerca gregaria* (Forsskål), bred at Tel Aviv University, School of Zoology. The animals were kept at high density: over 100 animals in cages of 60 l, under a 12 h:12 h light/dark regime, a temperature of 30℃, and 30-65 % humidity. Animals were fed daily with wheat seedlings and dry oats. All experimental locusts were the offspring of many generations of gregarious locusts reared in these conditions.

Experimental setup

A ring-shaped arena was used for the assessment of the behaviours of individuals and swarms. It comprised blue plastic walls (60 cm diameter and 55 cm high) with an inner central cone dome (30 cm diameter). The bottom of the structure was covered with a thin layer of Fluon (Whitford Plastics Ltd., Runcorn, UK), in order to prevent locusts from climbing. A white paper covered the floor of the arena and was changed before each experiment. Light sources around the arena lit its floor evenly. A video camera (Sony FDR-AXP35: 4K Ultra HD) was positioned above the arena and filmed the locusts throughout the experiment.

Monitoring the locust movements

Prior to the experiments, each of the experimented locusts was tagged with a unique barcode glued to the dorsal side of its prothorax using a drop of Epoxy glue. Using the video footage at 25/3 frames per second, the trajectory of each animal was reconstructed offline using BugTag software (Robiotec Ltd., Israel). To this we added a custom-designed multiple-target tracking and a trajectory-smoothing method (for details: Ariel et al., 2014a). briefly,

5

segments in which tags were not identified (less than 5 cm or 25 s) by the system positions were interpolated. We thus obtained the positions of the tags' centre of mass relative to the arena centre, at a final resolution of 2-3 pixels (ca. 0.5 mm), for about 99% of the video frames. In order to reach 100% recognition, we completed the analysis manually for the remaining frames. In three out of the 26 experiment the tracking system failed to recognise 1-2 animals in the grouping stage. Therefore, the order parameter was calculated based on the recognized animals only. These miss-identifications, which only slightly affect the order parameter calculated in the grouping stage, have no influence on any of the kinematic statistics in any of the stages (that only describe the focal animal).

Experimental procedure

In the morning of each experiment day, 10 locusts were taken from their cage and individually tagged. Thereafter, one locust was randomly chosen and placed in the arena alone for one hour. The other 9 locusts were then added for an additional hour; after which they were removed. The remaining locust was then filmed for another hour.

Analysed parameters

Following the recognition process, we analysed the middle 40 min of each of the 3 hours of the experiment using MATLAB (MathWorks, Natick, MA, USA). The following parameters were calculated:

1. Order parameter – the absolute value of the average direction of movement of the walking animals (clockwise denoted as -1 and anticlockwise as +1), averaged for all the frames.

2. Fraction of walking – the number of frames an animal walked divided by the total number of analysed frames.

3. Detection of walking bouts – a walking period was defined as walking at a speed above 0.25 cm/sec for more than one third of a second. This double threshold allowed us to overcome the limitation of the tracking system and avoid recognizing very small movements as real walking.

4. Speed – averaged for all experimental animals and analysed frames when the animal walked and the speed differed from zero.

5. Walking bout duration – the time an animal moved continuously between one pause to the next, averaged for all walking bouts.

6. Pause duration – the time an animal did not walk between one walking bout and the next, averaged for all pauses.

Statistical analysis

All statistical tests were conducted with MATLAB. To compare the order parameters, Wilcoxon rank-sum test was used. To compare among the three stages, Friedman Test was used. Since all comparisons were significant according to the latter test, Bonferroni multiple comparison post-hoc test accompanied each Friedman Test, in order to reveal the significantly different groups. The *p* values noted are of the Bonferroni multiple comparison post-hoc test. Correlations were conducted using Spearman's Rank Correlation test.

Modelling

We employed a two-dimensional model comprising N agents moving at a fixed speed v in a square domain of linear size L and periodic boundaries. Position and heading (direction) of each rectangle centre were denoted by $x_i(t)$ and $\hat{v}_i(t)$, respectively, where $x_i(t) \in [0, L]^2$ and $\|\hat{v}_i(t)\| = 1$. In addition, $w_i(t)$ is a Boolean variable that denoted whether at time t,

agent i is moving ($w_i(t) = 1$) or pausing ($w_i(t) = 0$). In order to take into account the physical size of animals, we assumed that each agent is a rectangle with sides $a_1 \times a_2$.

In each simulation step, the position of moving agents changed by amount v in the direction $\hat{v}_i(t)$,

$$x_i(t + 1) = x_i(t) + v w_i(t) \hat{v}_i(t) \quad (mod \ L).$$

The new velocity of agent i depended on three terms: i. Inertia (its own direction); ii. the direction of other agents in its receptive field (see below), possibly with a memory; and iii. a random vector with unit norm $\xi_i(t)$. The receptive field view is a circle around $x_i(t)$. Unlike previous models, we assumed that an agent sees all other agents whose rectangle is within a distance r from $x_i(t)$, and not merely the center of the rectangle. Denoting the four corners of the rectangle describing agent j as indexes $c_j^1(t), \dots, c_j^4(t)$, we defined the edges of the rectangle as the line segment $e_j^k(t) = [c_j^k(t), c_j^{k+1}(t)]$, where $c_j^5(t) = c_j^1(t)$. The adjacency matrix $A_{ij}(t)$ is a zero-one matrix that is one if agent j can be observed by agent i at time t, i.e.,

$$A_{ij}(t) = \begin{cases} 1 & \left(e_j^1(t) \cup e_j^2(t) \cup e_j^3(t) \cup e_j^4(t) \right) \cap \{x: \|x_i(t) - x\| \le r\} \ne \phi \\ 0 & \text{otherwise} \end{cases}$$

Then, the set of observable neighbours of agent i at time t as,

$$I_i(t) := \sum_{i \ne j} A_{ij}(t).$$

The average direction in the receptive field at time t, is $\hat{u}_i(t) = u_i(t)/\|u_i(t)\|$, where

$$u_i(t) = \left(\frac{1}{n_i(t)} \sum_{j \in I_i(t)} \cos(\hat{v}_j), \frac{1}{n_i(t)} \sum_{j \in I_i(t)} \sin(\hat{v}_j) \right).$$

Here, $n_i(t)$ is the number of elements in $I_i(t)$.

Overall, the new heading $\hat{v}_i(t+1)$ was the weighted average of four terms: inertia, direction of moving intersecting rectangles at time t, the average heading of moving intersecting rectangles at time $[t-5\ldots t-1]$, and independent random vector with unit norm $\xi_i(t)$ from a uniform distribution of $[-\pi, \pi]$. Angles of other agents to the focal agent were provided by the simulator. Let the set of the weights be $[q_1 \ldots q_4]$. The updated weighted averaged direction, was, therefore, $\hat{v}_i(t) = v_i(t)/\|v_i(t)\|$, where

$$v_i(t+1) = q_1\hat{v}_i(t) + q_2\hat{u}_i(t) + q_3\frac{1}{5}\sum_{s=1}^{5}\hat{u}(t-s) + q_4\xi_i(t)$$

The weights were set to $Q = [0.2, 0.2, 0.3, 0.3]$. Collisions of agents with each other were not taken into account, as our previous study found that visual stimuli is sufficient for generating collective behaviour (Ariel et al., 2014a).

Switching between walking and pausing was determined as follows. Assuming exponential distributions of moving and pausing durations, the probability to proceed moving was given by a single parameter, p_w, describing the probability per-step of an agent to continue walking, $p_w = P(w_i(t+1) = 1 | w_i(t) = 1)$. Similarly, $p_s = P(w_i(t+1) = 0 | w_i(t) = 0)$ was the probability per-step that a stopping agent will continue stopping.

The following statistics were calculated over steps 200-1000 of the simulation. Brackets denote averaging over all analysed frames.

1. Order: $\theta(t) = \frac{1}{N}\langle\|\sum_{i=1}^{N}\hat{v}_i\|\rangle$.

2. Spread: $S(t) = \frac{1}{N(N-1)}\langle\sum_{i=1}^{N}\sum_{j\neq i}|x_i(t) - x_j(t)|\rangle$

3. Number of neighbours: $n_i(t) = \frac{1}{N} \langle \sum_{i=1}^{N} I_i(t) \rangle$

4. Steps to regroup: the number of steps that passed from the first step in which an agent has no neighbours within its visual field $(n_i(t) = 0)$ until it encounters a neighbour again $(n_i(t + s) > 0)$.