博士論文

Experimental and Theoretical Analysis of Exogenous and Endogenous Bursts in Honeybee Hives

(セイヨウミツバチの外因性および内因性バーストの実験的/理論的解析)

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Abstract

Social entrainment of the animal grouping is important for the group survival. The synchronization of circadian rhythms in groups has attracted attention and studied for a long time. However, there are few reports on social entrainment on a smaller time scale than the circadian rhythm. By analyzing massive data set of the approximately 5,000 individuals tracking data of honeybees (*Apis mellifera*), we revealed that honeybees showed bursting behavior which is one of the social entrainment and the time-scale is smaller than the circadian rhythm regarding their locomotion activities. These bursts are quantitatively classified as endogenous or exogenous based on their evolutions of the global kinetic energy. The features of exogenous bursts were sudden increases in the global kinetic energy, which occurred because of external stimuli. On the other hand, the characteristics of endogenous bursts were gradual increases in the global kinetic energy, which happened spontaneously, potentially resulting from intrinsic bee interactions. Also, we demonstrate that physical contact is one of the media for the endogenous bursts by the empirical data and the simulation. These results suggest that the bees which become active before a peak of an endogenous burst trigger the endogenous burst and we named these bees "pioneer bee". Moreover, we demonstrate the pioneer bees are not identified randomly but categorized into two types. One is the bees related to the so-called foraging behavior that brings some information from the outside into the hive, and one is a non-forager bee that is enhanced by returned forager and spreads the information. These findings suggest the burst has a role in the function of spreading the external information through the hive and make the individuals behave as one integrated group.

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Chapter 1

Introduction

1.1 Synchronization

The synchronization of grouping behavior is essential for the daily survival of a group, as this practice enables the achievement of common goals, such as breeding, defense against predators, collective hunting, and gathering as a means of energy conservation. Groups of starlings and sardines is a well-known example of groups acting as super-individuals[3]. Grouping reduces the chance of predator attacks.

Drosophila increases the chance of mating by synchronizing its circadian rhythm with that of the group[4][5][6]. Meanwhile, honeybees perform the so-called waggle dance to announce the locations of feeding areas and to synchronize the global clock within a hive to transfer information[7].

How do groups of animals achieve such collective synchronization despite the typically different intrinsic clocks of individuals?

A widespread phenomenon in the synchronization of group time structure is the circadian rhythm. Research on the circadian rhythm has a very long history. Drosophila, social insects (such as ants and honeybees), fish[8], birds[9], and mammals[10][11] have been the target of circadian rhythm research. [1]

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[1]Social animals do not always show social entrainment. Furthermore, entrainment of the circadian rhythm has not been observed in social mammals even though they have social interactions[12], [13], [14].

1.2 Time in the honeybee society and their social entrainment

In general, a hive of honeybees contains one queen bee and thousands to tens of thousands of worker bees. They use the waggle dance to tell their hive-mates the source of food, feed them in groups, and store food in the hive.

A hive is made in a tree hollow. Thus, its inside is a constantly dark environment that cannot be penetrated by sunlight. Therefore, except for foraging bees, no individual knows the time outside the hive, and a global clock is shared within the hive to conveniently maintain efficient hive operation.

For example, the waggle dance is a widely known language for communicating the positions of food outside hives. The direction of the head and the angle of gravity direction correspond to the direction angle of the brooding area and the direction of the sun, respectively. For this language to have an accurate meaning, the direction of the sun or the time of dancing must be sensed. In fact, bees can share information about brooding areas correctly using the waggle dance even without seeing the sun[19]. Meanwhile, foragers transfer nectar to a specialized group called nectar receivers, which accepts and then stores the nectar. Although nectar receivers stay inside the constantly dark hive, they need to adjust their circadian rhythm to that of the foragers. Thus, in the life of social groups, such as honeybees, the global temporal coordination of activities seems to be important for maintaining efficient hive function and hence hive fitness.

How do honeybees ' hives generate collective time?

1.2.1 Southwick and Moritz's experimet

In 1987, Southwick and Moritz reported that honeybees have a mechanism for synchronization among individuals [20].

First, they collected 50 bees from a honeycomb and assigned these to one group. The authors raised Group A under the same light and dark environment (LD condition) as that outdoors and raised Group B under DL condition (opposite that in the field). The total activities of each group were measured by the oxygen concentration in the breeding box, and the findings confirmed a half-day phase shift in the activity peaks of each group. Thereafter, these groups were transferred to a dark environment, and two different settings were prepared. In Setting C, bees in Groups A and B were able to keep contact over the net. In Setting D, bees in Groups A and

B were separated spatially, and only air could be exchanged through the pipe connecting the breeding boxes. Then, the oxygen concentrations were monitored for Settings C and D.
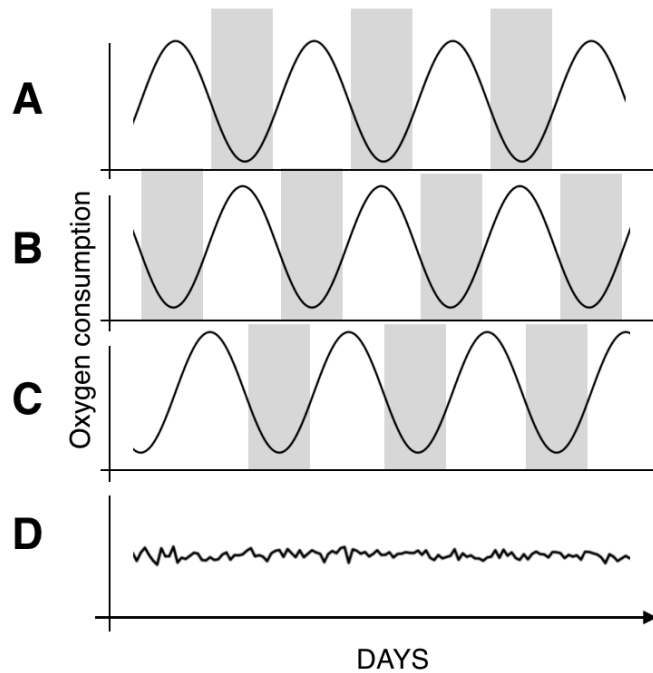
Figure 1.1: Results of Southwick and Moritz's experiment. Typical oxygen consumption rhythms. Shaded areas represent times of subjective night. (A) Metabolic rhythm of bees taken from natural LD condition. (B) Metabolic rhythm of bees taken from DL condition. (C) Metabolic rhythm of mixed group of LD and DL bees with contact. (D) Mixed group without direct contact.

According to the findings, under Setting C, the activities of Groups A and B were synchronized, and the cycles of oxygen concentration shifted and peaked at half the original cycle peaks of the two groups. Meanwhile, in Setting D, no clear pattern was observed in oxygen consumption (Fig. 1.1).

The results showed that the bees contacting directly through the net showed synchronization of their activity cycles, and the bees contacting via air became irregular in groups. In other words, the result suggested that bees use direct contact or vibrations, rather than volatile substances, to synchronize activity levels among individuals.

In the discussion of this paper, the authors stated that bees' trophallaxis (mouth-to-mouth interaction for food or chemical transfer) is essential for activity synchronization. Korst *et al.* found that trophallaxis provides the

major communication mechanism among honeybees[21]. Such interaction-dependent synchronization may be the candidate synchronization of individual circadian rhythms. However, the same authors revealed in 1994 that the trophallaxis is not essential in the synchronization of at least the circadian rhythm[22].

1.2.2 Friesch's experiment

In 1994, Friesch *et al.* brought wild honeybee colonies into a laboratory; they studied the circadian rhythms of the hives and of individual bees isolated from the hives[23]. The results revealed that the circadian rhythms of the isolated bees drifted from those of their colonies, with the former's periods being either longer or shorter than those of the latter. After 12 days of isolation, the phase coincidence between the circadian rhythms of the isolated bees and of the hives was lost. The authors concluded that the circadian rhythms of the bees in the hives were caused by a social synchronization of the rhythms of the individual bees.

These findings suggested that honeybees form a collective time structure that is independent of their individual time structures.

1.3 How do honeybees synchronize and generate their own time?

Bloch *et al.* presented four hypotheses about the possible mechanism for the entrainment of the circadian rhythm in honeybee colonies(Fig. 1.2)[1].
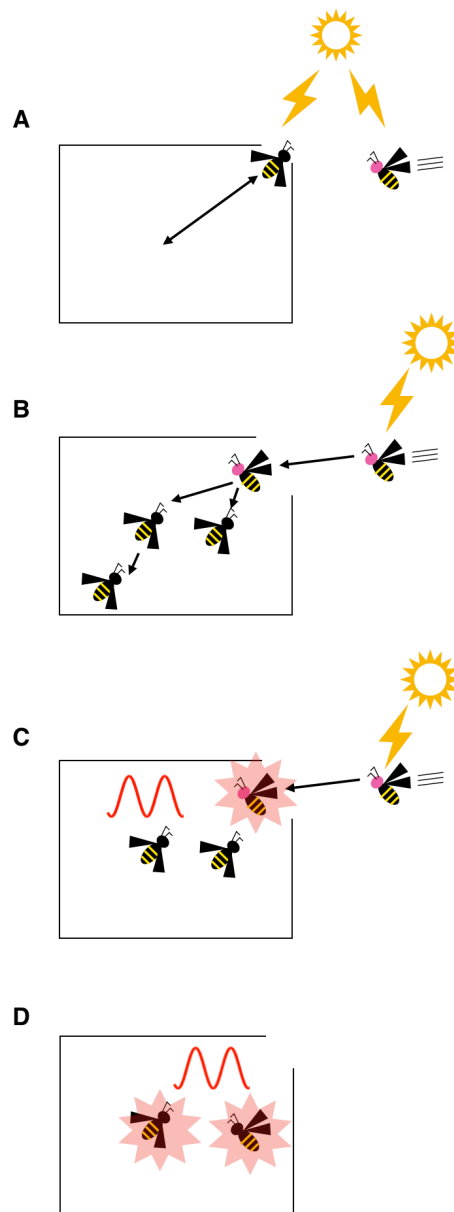
Figure 1.2: Possible mechanisms of entrainment of circadian rhythms in honeybee colonies proposed by Bloch (2013)[1]. The titles of each hypothesis are quoted from the original paper. (A) Entrainment by environmental zeitgebers: Both foragers and hive workers synchronize directly with sunlight and temperature cycles. Foragers can be easily entrained by the cycle of sunlight because they spend a large part of daytime outside the hive. Meanwhile, hive bees may experience sunlight and temperature cycles when occasionally approaching the hive entrance. (B) Social entrainment by direct contact: Environmental zeitgebers entrain foragers, who then entrain hive bees through physical or short-distance interactions. The entrained hive bees socially synchronize hive bees that have not encountered foragers. (C) Entrainment by environmental cycles driven by forager activity: Foragers change the hive environment, which in turn entrains the rhythm of other bees in the hive. (D) Self-organized social synchronization: Active bees change their environment, and the sum of all personal activities forms a weak environmental vibration accompanied by an inadequate number of bees. As the number of bees synchronizing in phase increases, the amplitude of these vibrations rises and eventually becomes strong enough to tune all the honeybees in the hive.

The mechanism of the first hypothesis does not considerably differ from that of solitary insects usually accompanied by strong variations of ambient light and temperature, and this hypothesis is not consistent with the observation of the honeybee hives in the aforementioned study.

Each of the three remaining hypotheses was supported by a different study conducted in the past. However, we postulate that no complete answer exists. The mechanism by which the circadian rhythm was shared among the bees may be understood/interpreted by combining more than two of the above hypotheses.

So far, by reviewing previous studies on circadian rhythms, we have seen how honeybees produce a common/collective time structure. However, research has been actively conducted not only on honeybees but also on certain kinds of rodents[24] and bats[25]. When common time structure is obtained in an organism, can temporal periodicity other than the cycle of the sun be acquired?

1.4 Synchronization and its importance in our brain

In our brain, a large amount of periodic activity is produced by neurons on a time scale smaller than the diurnal cycle[26][27]. These periodic activities derive from not only responses to external stimuli but also so-called spontaneous activities that regularly carry out the firing activities without input from the outside[28][29].

Spontaneous activity was once believed to be random[30]; however, recent studies suggest that it shows burst synchronization[31][32].

Izhikevich and colleagues constructed a large artificial neural network consisting of 100,000 model neurons that are faithful to detailed anatomical findings in cortical neural circuits[33][34]. Although individual nerve cells initially showed random activities, they gradually began to repeat specific activity patterns. This result suggested that the neural circuitry can organize activities on its own.

What is the function of burst synchronization for the brain?

In general, the dynamics of an individual neuron is stochastic and unstable. By mathematical calculation, Diesmann *et al.* stated that synchronization of individual neuron activities would enable these circuits to be stably propagated while maintaining time accuracy[35]. This phenomenon is assumed to play a role in stabilizing the behavior of cortical circuits.

Considering the brain as a group of a large number of individual neurons,

we suppose that the phenomenon of synchronization seems to be used for groups. Moreover, this synchronization occurs without any external input and is hence a truly collective time structure. The brain has one conscious state despite comprising a massive number of neurons. Thus, given that a biological group successfully uses synchronization to maintain the order of the group system, such as the brain, we may believe that the biological group functions as a super-individual that is beyond the simple sum of its constituents.

1.5 Scope of this thesis

In this thesis, we regarded the honeybee (*Apis mellifera*) as a representative of organisms that gather in large numbers to form groups because they are one of the most popular animals that exhibit social entrainment.

Our collaborator Gernat *et al.*(2018) developed a high-throughput automatic monitoring system for honeybee hives. By attaching a "bCode" device (a custom matrix barcode) to the thorax of every individual bee in a hive, they successfully tracked each bee's positions, speeds, and orientations using recorded digital images. Gernat *et al.* used the same tracking system to study bees' trophallaxis networks and calculate their communication frequency. They found that the distribution of communication intervals follows a power law on a scale shorter than 100 seconds. In other words, bees communicate in a temporally intermittent manner which the authors called bursts and which considerably resembles human communication networks.

Using the same massive dataset as that in their research, we developed a complementary analysis that examined a different bursting behavior: the bursts of locomotion activities that occur in beehives.

In this thesis, we analyzed and discussed the following questions.

1). How do the bursting behaviors of honeybees develop?

2). What is the cause of the individual-level activity that is transmitted through the hive?

3). What personality or characteristics of bees trigger a global bursting pattern?

In Chapter 2, we discuss how the bursting behaviors of honeybees develop. In Chapter 3, we present the results of an agent-based model and compare them with those of an actual dataset. In Chapter 4, we reveal the characteristics of individuals that trigger a global bursting pattern. Finally, in Chapters 5 and 6, we summarize this study and propose research prospects.

Chapter 2

Analysis of exogenous and endogenous bursts in honeybee hives

2.1   Introduction

Regarding the time synchronization (social entrainment) in biological groups, the synchronization of circadian rhythms in groups has attracted attention and studied for very long time. As a mechanism by which this circadian rhythm synchronization occurs, Bloch *et al.*(2013) introduced four hypotheses as seen in the previous chapter. However, there are few reports or studies on social entrainment on a smaller time scale than the circadian rhythm.

Social insects have been extensively studied, including their collective society dynamics, the individuals constituting their societies, and the relationships between individuals[36][37]. Regarding individual divisions of labor, for example in ant or bee societies, it has been found that worker individuals will swap between foraging and nursing roles, and it has been suggested that the division of labor is not determined by some central control system but by stimuli from the external environment and/or by interactions between individuals[38][39].

In recent years, thanks to the development of individual tracking technologies, empirical studies which track and analyze all the individuals constitutes the group has increased. Mersch *et al.* (2013) tracked hundreds of individual ants and found that the spatial distribution of individuals was responsible for their divisions of labor. Also, Crall *et al.* used an automated tracking system to monitor nest behavior and foraging activity of entire bumble bee (*Bombus impatiens*). The author showed that most foraging is performed

by a small number of workers and that the intensity and distribution of foraging is regulated at the colony level in response to forager removal. The author showed that the bees who tend to stay in the central part of the nest have a relatively high possibility of replacing to forager bee when the nest is disturbed.[40]

In this chapter, we focused on the phenomenon that honey bees synchronize activity in groups (hereinafter, this is called "Burst"), and analyzed how this phenomenon occurred and was attributed.

2.2 Data/Analysis

2.2.1 Data

All data were provided by G. Robinson and his group from the University of Illinois; their detailed data collection methods are reported in their paper[41]. To summarize briefly, they used a single cohort hive consisting of 1,200 1-d-old adult worker bees and one unrelated, naturally mated queen. This kind of colony commonly is used in experiments that deal with division of labor in order to control the effect of age, and instead focus on the behavioral development of worker bees as a function of their genetics and social interactions.
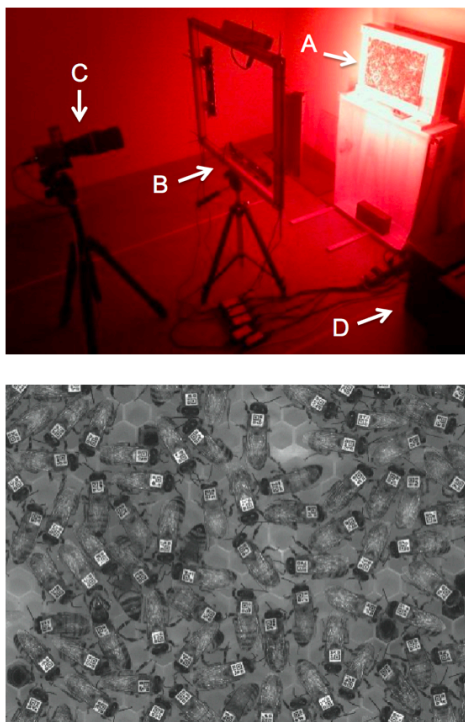
Figure 2.1: The experimental setting. Top: (A) The hive was in a small glass-walled observation case which held a single honeycomb. The hive was designed to prevent bees from crawling over each other and to ensure maximum exposure to the camera. The hive had an exit to enable bees to forage outside normally. (B) The hive was illuminated by infrared lights which bees cannot detect to ensure natural behavior. (C) Bees were imaged with a high-resolution machine vision camera (Allied Vision Technologies Prosilica GX6600). They recorded one image per second continuously throughout the experiment. (D)The images were stored on a disk array. Bottom: The example image obtained from this system, showing barcoded bees inside the observation hive. These photos were provided from Tim Garnat.

Each colony was provided with the same amount of honey and artificial "bee bread" (a mixture of 10 parts pollen, 9 parts honey, and 1 part water). Their method used custom 2D QR barcode devices, called "bCode." These devices were attached to the thoraxes of individual bees and provided sequences of digital images that enabled reliable identification and tracking of every individual in the hive (Fig.2.1). The digital images were converted into data on the coordinates (x, y) and angle of each bee for every second, with x ranging from 0 to 6,576 px and y from 0 to 4,384 px. The hive was installed vertically in a dark room with constant temperature(approximately 35°C) and humidity and was connected to the outdoors through a tunnel whose entrance was closed until two or three days after the start of the ob-

servation and then opened to allow workers to exit the hive and to begin
foraging. These bees provided sufficient honey to feed the entire hive for the
duration of the experiment and enough bee nutrition for 2 days. They could
not track some bees because of deaths of the bees and sublations of "bCode".
They removed these un-trackable bees before the entrance was opened from
the tracking data. Their software can identify bees in 94% (determined by
manual analysis of 60 images) of all cases with an error rate of 1.4% (de-
termined by manual analysis of 5,000 detected barcodes). There were five
separate trials (five different hives) of the experiment over the July of 2012
and 2013 (Table 2.1).

Data name	Experimental date	The number of glass clearing	Total number of bees	The number of detected foragers	The number of detected bursts
1201	2012/7/4 ~ 2012/7/12	12	925	214	93
1202	2012/7/18 ~ 2012/7/24	13	946	203	40
1203	2012/7/28 ~ 2012/8/3	12	958	227	59
1301	2013/7/3 ~ 2013/7/11	13	1165	246	72
1302	2013/7/16 ~ 2013/7/24	13	965	251	65

Table 2.1: Detailed information of the experiments.

Detection of "forager"

After the entrance was opened, bees often went to the outside. It is well
known that bees go outside as a "foraging" or an "orientation flight". Al-
though when to become forager is variable, under normal colony conditions,
forager bees are workers with an age of over three weeks, and they shift to
perform outside tasks including water, nectar, pollen or resin collection[44].
In order to become foragers, they need to know the landscape around their
hive and the position of the hive entrance. They do so by taking orientation
flights in the days preceding their first foraging flights. Young bees walk out
of the hive, fly a short distance around their hive. After a few minutes, the
bee returns to its hive without carrying any pollen or nectar[45]. Because the
time durations of our experiments are within a week (namely, shorter than
three weeks) and the experimental duration and bees' age are almost same,
there is no "real-forager" in our experiments. However, we can consider bees
frequently attend to the orientation flight are as "forager-candidate" which
can become real forager after three weeks. In this study, we identified bees
that went outside for at least 30 minutes or more every day were identified
as "forager-candidate" (potential forager) (hereinafter, this is called forager)
in order to distinguish just the orientation flight[1].

Detection of "trophallaxis"

Bees often exchange food and this can also serve as a communicational
function. This " mouth-to-mouth " contact communication has been called
"trophallaxis". An image recognition algorithm was used to detect trophal-
laxis. Namely, it was identified as occurring when each proboscis was con-
nected (For details, see [41]). The detected trophallaxis events were then
analyzed by a weighted undirected network, where individuals represented
by nodes and the duration of trophallaxis events represented by edges.

Detection of "waggle dance"

By performing waggle dance, foragers can share directions and distance to
patches of flowers and water sources with other nest-mates[46][47]. The danc-
ing bees waggles back and forth as they move forward in a straight line, then
round to repeat the dance. Our collaborator Weibing DENG developed an
algorithm for the detection of the waggle dance. The rotation of the bee was
used as an indicator of the waggle dance.

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[1]As another identified option, we can identify, for example, top 10% bees that went
outside frequently as " foragers. " We also tried to use these forager list for the analyzing
but the results were almost the same.

Orientation of the bee at time t is:

$$\boldsymbol{n_t} = (n_{x_t}, n_{y_t}) \tag{2.1}$$

where n_{x_t}, and n_{y_t} denote the respective x component and y component of the head direction of each bee. and the scalar product of $\boldsymbol{n_t}$ and $\boldsymbol{n_{t+1}}$ is:

$$\boldsymbol{n_t} \cdot \boldsymbol{n_{t+1}} = n_{x_t} \cdot n_{x_{t+1}} + n_{y_t} \cdot n_{y_{t+1}} \tag{2.2}$$

If $\boldsymbol{n_t} \cdot \boldsymbol{n_{t+1}} < 0$ then we could say that the orientation of the bee changes more than 90 degree. As the first condition to assume that the bee is dancing, they used following condition: In the five consecutive seconds, there should be at least four negative scalar product, which means, in 5 consecutive seconds, the potential dancer has to change its orientation at least four times more than 90 degree. Also, as the second criterion, the exterior product was used as:

$$\boldsymbol{n_t} \times \boldsymbol{n_{t+1}} = \begin{vmatrix} n_{x_t} & n_{y_t} \\ n_{x_{t+1}} & n_{y_t} \end{vmatrix} = n_{x_t} \cdot n_{x_{t+1}} - n_{y_t} \cdot n_{y_{t+1}} \tag{2.3}$$

If $\boldsymbol{n_t} \times \boldsymbol{n_{t+1}} > 0$ then the orientation of the bee make right turn. When the orientation change of the bee alternate between left turn and right turn in the five consecutive seconds, they identified the bee as "potential dancer". We identified that the bees which matched both conditions as "dancer".

2.2.2 Analysis

Indicator for the activities of the honeybee

To visualize and analyze the activities of the individual bee's and the entire hive, we defined the individual bees ' kinetic energy K as follow:

$$K_i(t) = \Delta x^2 + \Delta y^2 \,, \tag{2.4}$$

where Δx, and Δy denote the respective displacement of x and y coordinates of each bee per second (i). The hive activity level $K_G(t)$ was defined as the mean kinetic energy:

$$K_G(t) = \frac{1}{n} \sum_{i=1}^{n} K_i(t), \tag{2.5}$$

We used $K_G(t)$ as the indicator for the activities of the honeybee. Figure 2.2 gives an example of its evolution over time. As shown in Figure 2.2, the time series of $K_G(t)$ often indicated a sudden increase. We call such behavior a "burst", and it reflects the collective activation of individual honey bees' activities.

Detection of "burst"

We need a criterion to detect and decide the bursting phase. Kleinberg's burst detection algorithm was used to detect bursting events[48]. It assumes that the intervals of the events occur independently according to the following exponential distribution $f(x) = \overline{\lambda}e^{-\overline{\lambda}x}$. Here $\overline{\lambda}$ is the mean frequency. x is the interval between consecutive events. $\overline{\lambda}$ is defined as N/T, where N is the total number of events over the time series and T is the total length of the time series. Bursts are detected by comparing this expected frequency with the actual event frequency observed within the specific time window.
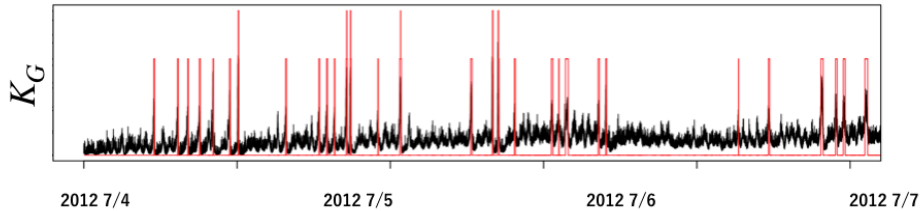
Figure 2.2: Example of the time series of $K_G(t)$. Black line describes the original $K_G(t)$ and the red bars describe the detected bursts. The height of the red bars indicate the burst level bl.

We define the burst level at every time point t of individual events. The burst level is expressed as $bl(t)$. When the burst level $i(t)$ is larger than or equal to 1, we consider it is as burst. When the local event frequency at time t shown as λ exceeds a certain threshold, the burst level can be updated. When λ_t exceeds $\overline{\lambda}s^1$, the burst level $bl(t)$ becomes 1 from 0. In the same fashion, if the λ_t exceed $\overline{\lambda}s^2$, $bl(t)$ becomes 2 from 1, and so on. We use $s = 2$ so that the burst level increases by 1 when the frequency doubles the previous one.

If we follow this process naively, the algorithm would detect a large number of bursts when the actual frequency of events fluctuates around the boundary between two burst levels 0 and 1. To alleviate this, another parameter γ is introduced into the algorithm. We use $\gamma = 1$ (see reference [48] for the details). We defined the "burst period" as a period with the burst level is larger than or equal to 1.

This algorithm is originally required the sequence of time stamps of events in question. However, our data is provided as a time series of K_G. So, Here, we consider K_G as frequency-based sequence and we need to convert our frequency-based data into the interval-based data. We simply calculate the

inverse number of the frequency as the interval between events.

As we will describe Results section, we divide the bees into the active states and inactive states in terms of their K_i. We will confirm that the number of active bees are more than 50% of the every bee in the peaks of the detected bursting phases.

Classifying bursts by their time evolution

To quantify the similarity between the time series, we used the dynamic time warping (DTW) method[49]. This algorithm compares the distances between points in two time series in a "round-robin" fashion, and then detects the shortest distance between sequences, and compares the similarity between the time series by using the shortest distance (i.e., the DTW distance).

2.3 Results

2.3.1 Basic observation

Individual observation

To quantify the activity of the bees, the individual kinetic energy $K_i(t)$ was taken as a characteristic measure. Figure 2.3 shows the example of $K_i(t)$ defined in (2). We can qualitatively see the difference between the forager and the hive bee in terms of the time evolutions of K_i.
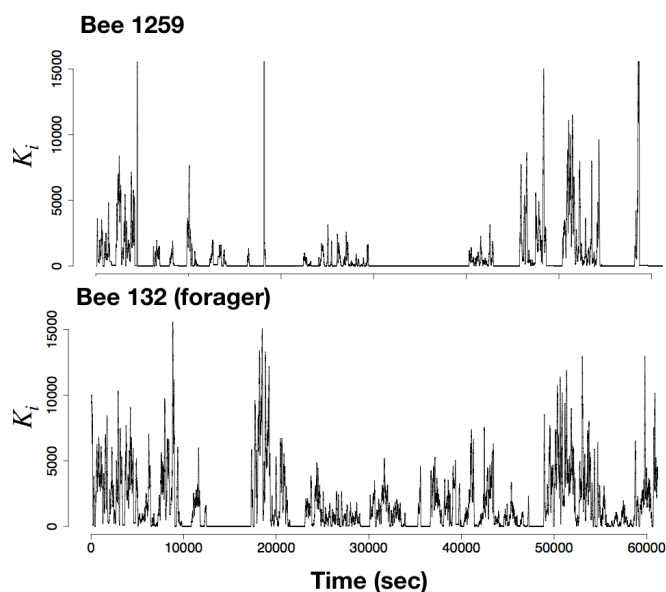
Figure 2.3: Example of K_i of the honeybees.

Figure 2.4 show the differences between the detected forager and non-forager bees in more detail. We only used the absent period from the hive to identify bees that went outside for at least more than 30 minutes were identified as forager. There are also significant differences between forager and non-forager in terms of average K_i, the average trophallaxis duration, and total dancing duration.
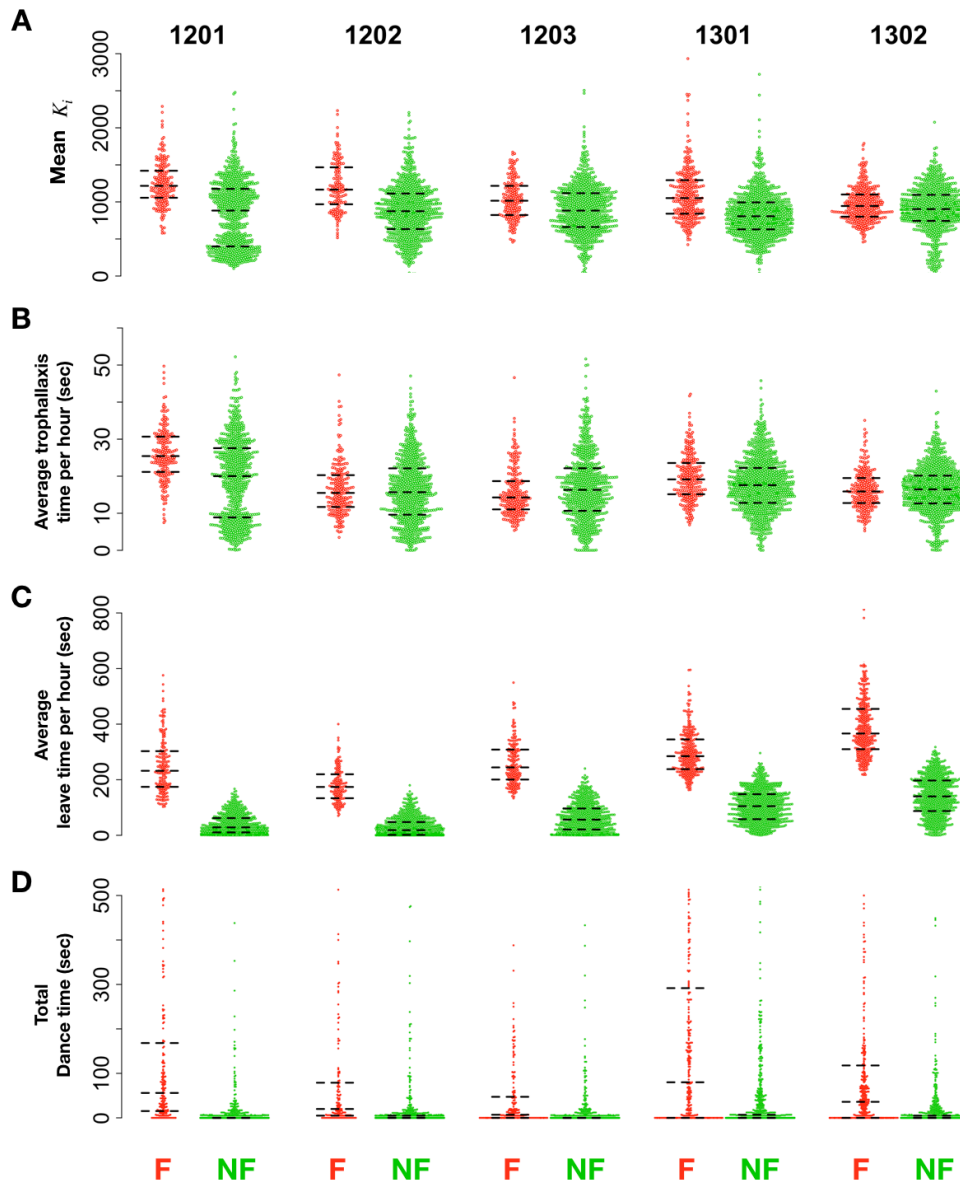
Figure 2.4: Differences of the distribution of the observed values between forager and non-forager. Beeswarm plots of average K_i(A), average trophallaxis time per hour (B), average leave time per hour (C), total dance time (D). ("Beeswarm plot" is a one-dimensional scatter plot. This plotting method emphasizes individual points in a distribution instead of binning them like a histogram.) The points show data of each individual bee, and thick lines show 75%, median and 25% values (from top to bottom) for each group.

The distribution of K_i is confirmed that the bees have a peak in the distribution of their kinetic energy(K_i). Especially, the typical a day time

distribution follows a bimodal distribution (Fig. 2.5). The similar result
was found in the ants activities[50]. This result suggests the bees' motions
are divided to "active state" and "inactive state". We will use this result
at Section2.3.3 to demonstrate that the physical/close distance interaction
between individuals is one of the cause for the bursts.
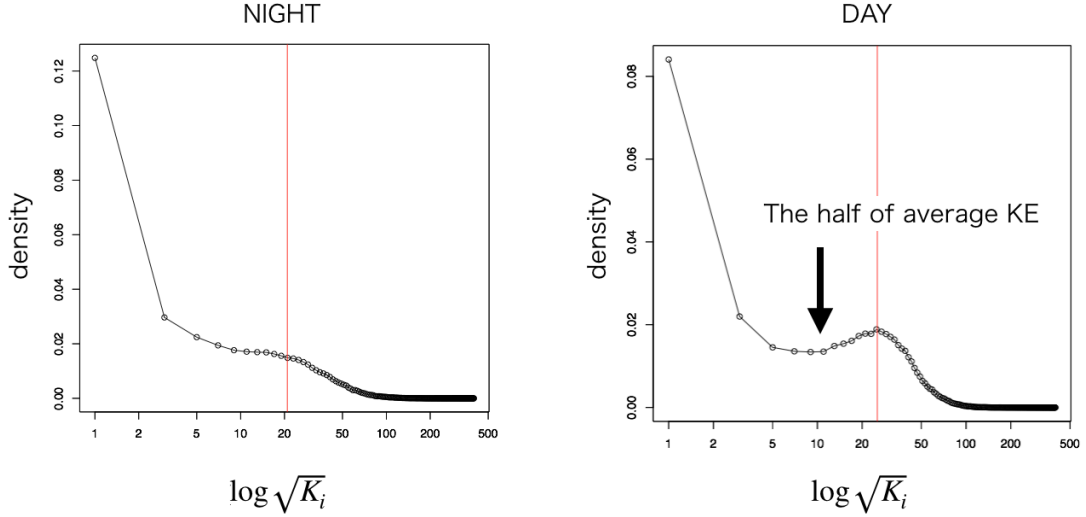
Figure 2.5: Distribution of the $\log \sqrt{K_i}$. Left: Night time(8pm - 4am). Right: Day
time(4am - 8pm). The red line depicted the average of $\log \sqrt{K_i}$.

Global observation

The global kinetic energy $K_g(t)$ was taken as a characteristic measure of the
beehive(Fig. 2.6). We found that the bee hive occasionally showed bursting
behavior. Since the bursting was observed in all trials, it is suggested that
the bursting is not a peculiar behavior.

Figure. 2.7 show ,after the entrance was opened, the cycles of K_G, the
global average out-of-hive duration and the global average dance duration
were correspond with diurnal cycle, although the cycle of the global trophal-
laxis duration was not correspond with diurnal cycle.
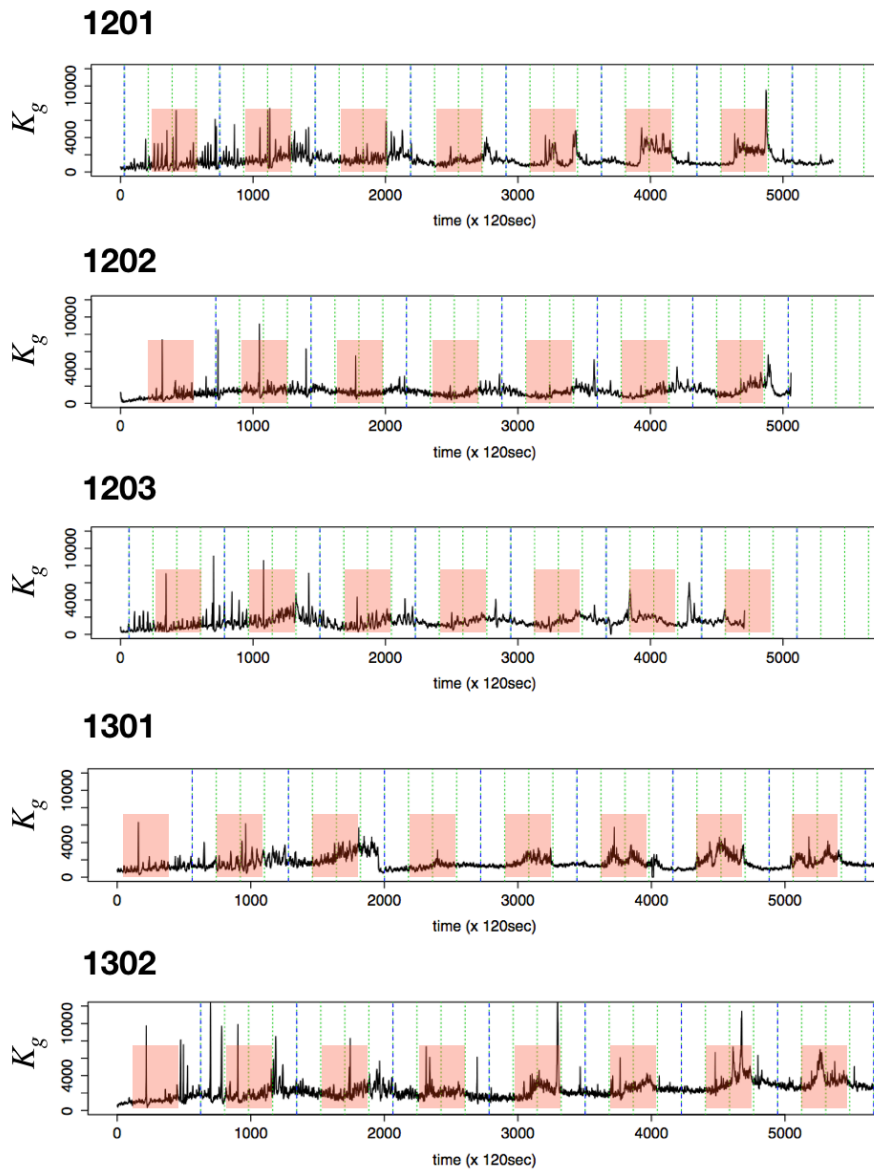
Figure 2.6: All time series of $K_G(t)$. The part shaded in orange represents the day-time(4am - 8pm).
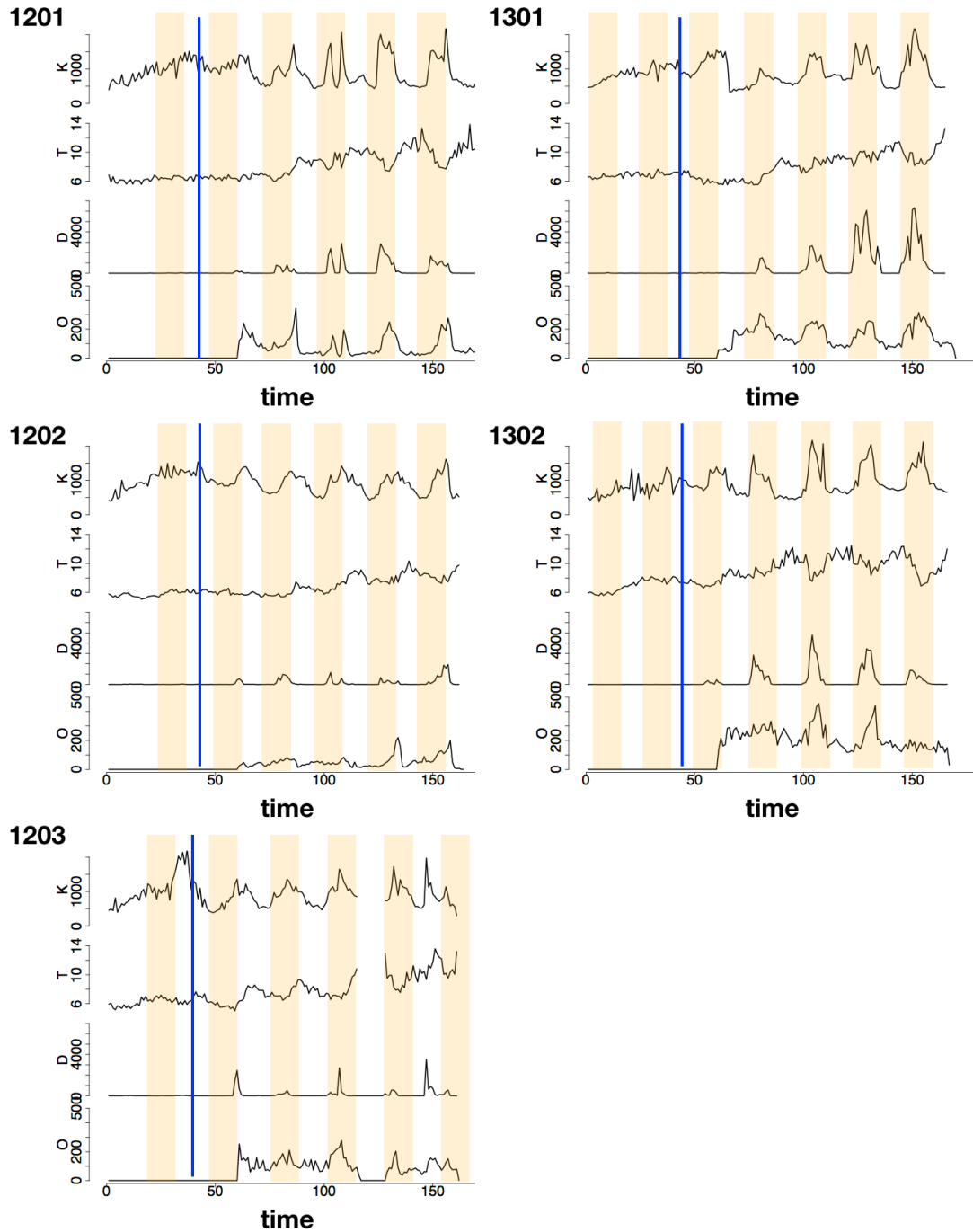
Figure 2.7: All time series of $K_G(t)$(K), global average trophallaxis duration per hour(T), global average dance duration per hour (D) and global total out-of-hive duration from the hive per hour(O) of every trial. The part shaded in orange represents the daytime. The time when the entrance was opened is depicted by the blue lines. In this figure, the time bin of x-axis is a hour. Note that our collaborator team did not get the data at the might of the day 5 of Trial1203 because of the error of the camera.

The occurrence of bursts was not periodic, and their distribution approximately obeys a power law (Fig. 2.8). Our collaborator Gernat *et al.* demonstrated that the distribution of communication intervals of honey bees followed a power law. This result suggests that global bursts do not happen randomly but under a kind of rule related to the trophallaxis network.
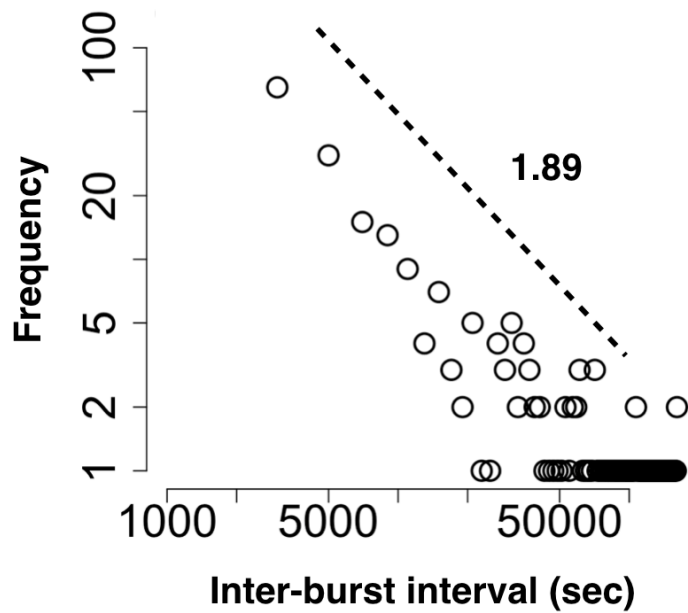
Figure 2.8: Distributions of inter-burst intervals among every trials obey power law like distribution. Dotted black lines indicate power law with exponent =-1.89. A power-law distribution is fitted with maximum likelihood methods as introduced by Clauset[2]. The p-value> 0.647.

Fig. 2.9 shows every distribution did not obey gaussian distribution. There are no significant difference of the distributions of K_G among trials, however, that of the trophallaxis durations and the out-of-hive durations are significant difference. The five data collections performed by Gernat *et al.* were the same experimental environment, but the periods of the experiments and the individual compositions of the hive differed for each trial. Under such conditions, it is a problem whether each trial can be regarded as the same. The result suggests that, at least regarding of the bee's kinetic energy, we can identify with each trial. We speculate that the differences of the trophallaxis duration and the out-of-hive duration are dependent on the constituents of each hive.
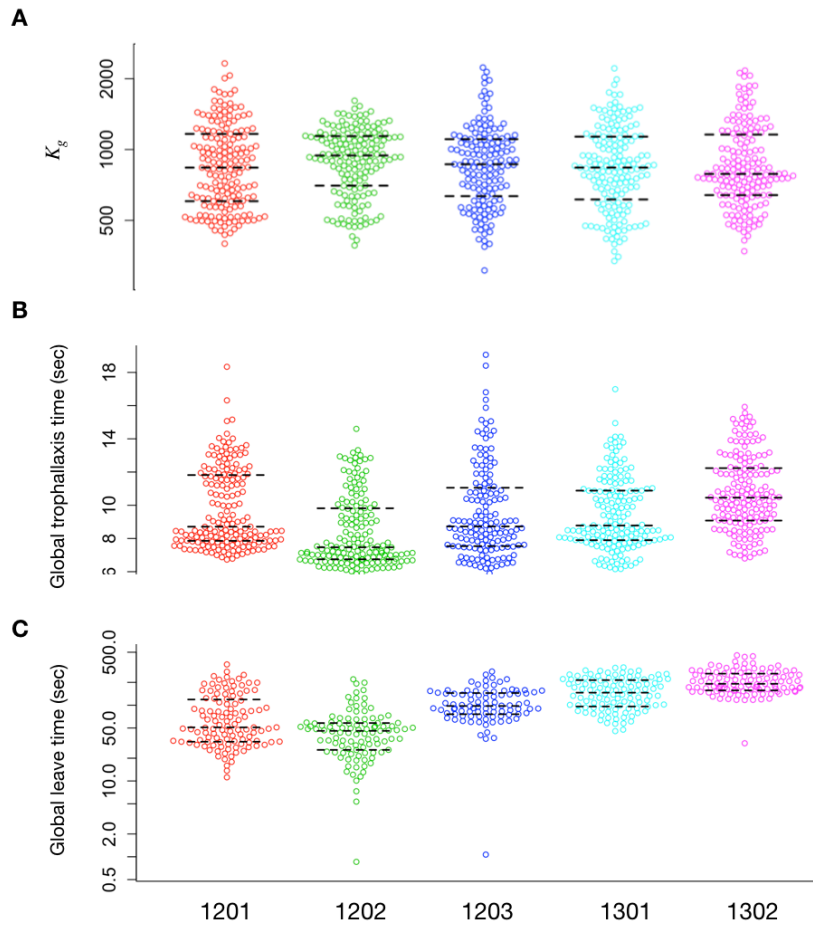
Figure 2.9: Differences of the distribution of the observed values among each trial. Beeswarm plots of average K_G per hour(A), average global trophallaxis duration per hour (B), average global out-of-hive duration per hour(C). Each color compatible with each trial. ("Beeswarm plot" is a one-dimensional scatter plot. This plotting method emphasizes individual points in a distribution instead of binning them like a histogram.) The points describe data of the average of each observed values every hours, and thick lines show 75%, median and 25% values (from top to bottom) for each group. Kolmogorov-Smirnov test A: p-value = 0.0097, B: p-value $< 10^{-8}$, C: p-value $< 10^{-4}$, Steel-Dwass test: (A) p-value = 0.31 (B) p-value $< 10^{-15}$, (C) p-value $< 10^{-15}$

2.3.2 Endogenous and exogenous bursts

The experimental hive was located in a dark, quiet place and its glass window was changed the window daily in the morning and at night to ensure a high detection rate throughout the experiment; this glass cleaning event often caused bursting behavior in the hive. However, bursts also spontaneously happened without glass cleaning events. We, therefore, classified each burst either endogenous or exogenous; this was determined by the temporal dynamics of each burst 's kinetic energy.

In Figure 2.10 and 2.12, we have overlaid the temporal evolution of K_G of endogenous and exogenous bursts in one graph. The blue and sky blue-hued curves represent endogenous bursts, which we assume were caused by intrinsic interactions among the bees. The slopes of the fitted curves of these bursts are fundamentally near to 2.0. On the other hand, the curves in red and pink represent the exogenous bursts which correspond to the glass cleaning events. The slopes of the fitted curves of these bursts are more than 2.3. Note that there were no burst the slopes of which were more than 2.3 except for the burst result from the glass cleaning events. Also, bursts often occurred when forager bees returned to the hive; these were classified as endogenous bursts since they resulted from intrinsic bee behaviors and relied on bee-to-bee communication. Also, the tilts of the fitted curves a of these forager related bursts are smaller than 2.2.

Figure 2.11 shows the active bees increase gradually at the endogenous bursting phase. On the other hand, the active bees increase suddenly at the peak of the endogenous bursting phase.
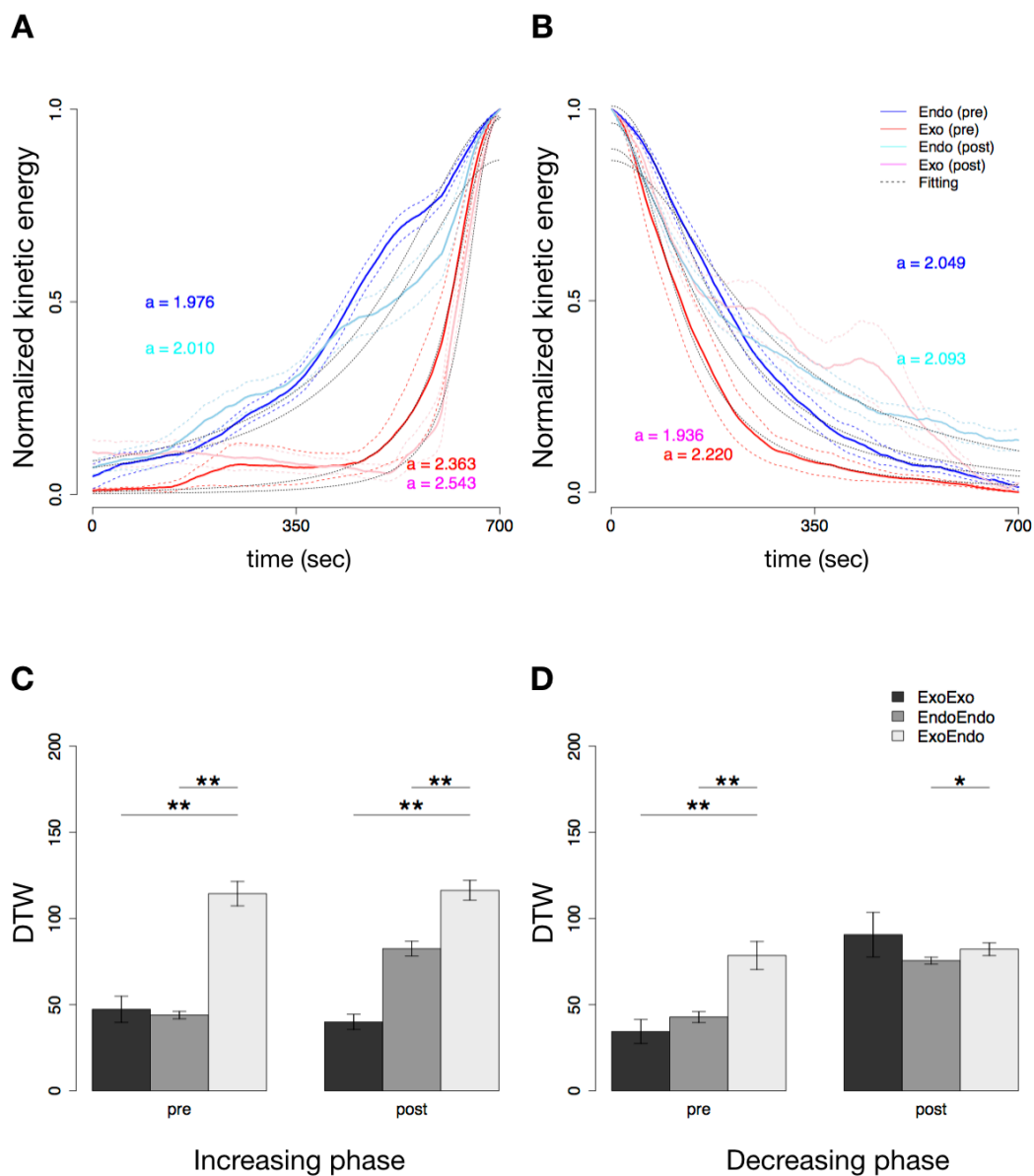
Figure 2.10: Endogenous burst and Exogenous burst are different in terms of their time evolution. (A,B) The time evolution of bursts in the increasing(A)/decreasing(B) phase of Trial 1201. Bursts due to an external perturbation are depicted as red (pre-entrance open) and pink (post-entrance open) lines, and bursts due to an intrinsic cause are depicted as blue (pre) and sky blue (post) lines. The dotted colored lines are the standard error for each time series. The K_G of all bursts are normalized into a range of $0 \sim 1$. The black dotted lines are fitted curves regressed by $K_G = c/(b + |t|^a)$. (C,D) Statistical analysis comparing the increasing/decreasing phase of each burst by DTW distance. (C) the increasing phase. (D) the decreasing phase. "Pre" refers to before the hive entrance opens, and "post" refers to after the hive entrance opens. EndoEndo compares endogenous bursts; EndoExo compares endogenous and exogenous bursts; ExoExo compares exogenous bursts. The p-value was determined by Steel-Dwass test. $* < 0.1$, $** < 0.05$
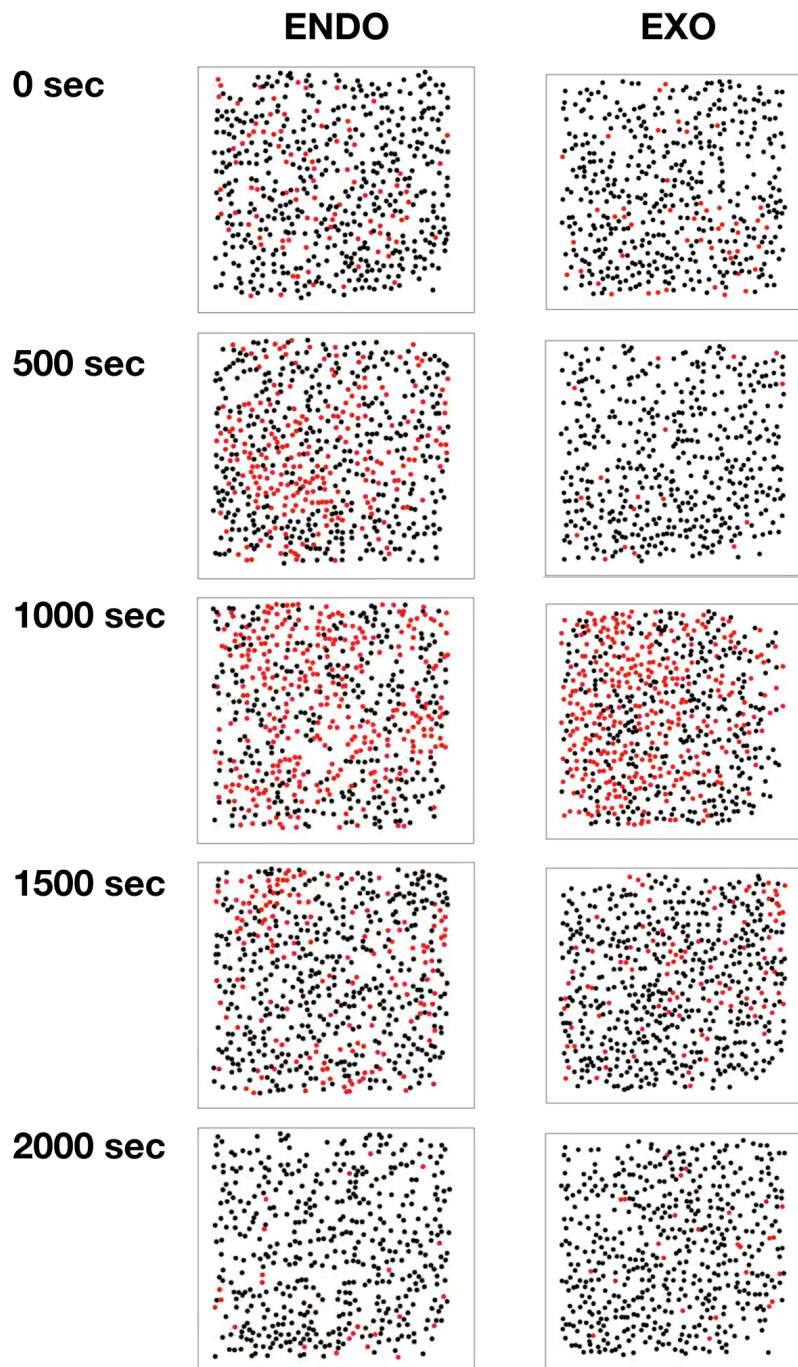
Figure 2.11: Snapshots of the typical spatiotemporal evolutions of the bursting behavior in the hive. "1,000 sec" corresponds to the peak of the bursts. The time sequence is from the top to the bottom. Left figures describe endogenous burst and the right figures describe exogenous burst. The red dots describe the active bees and the black dots describe the inactive bees.

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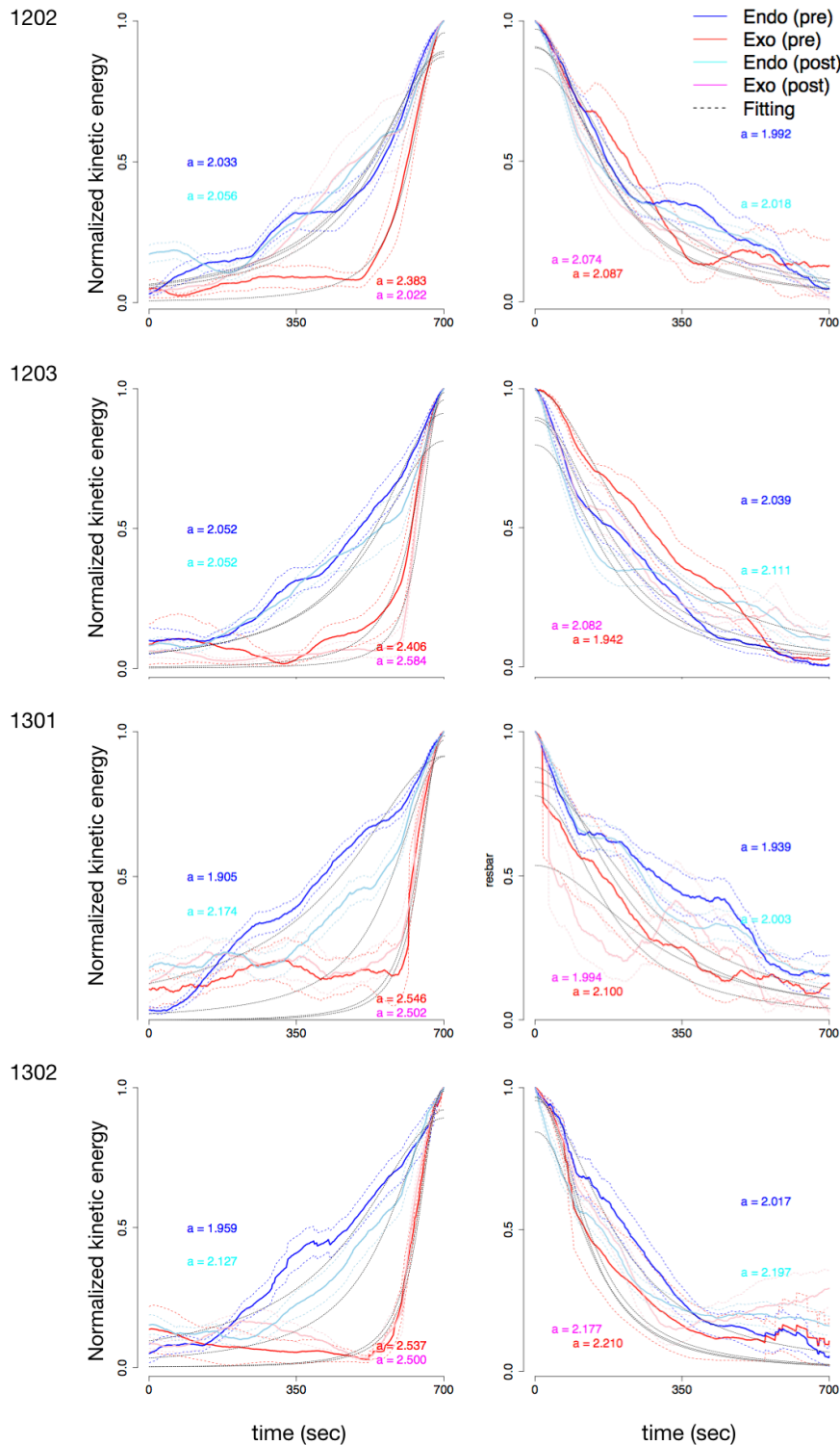
Figure 2.12: Time evolution of bursts in the increasing(A)/decreasing(B) phase of all trials. Bursts due to an external perturbation are depicted as red (pre-entrance open) and pink (post-entrance open) lines, and bursts due to an intrinsic cause are depicted as blue (pre) and sky blue (post) lines. The dotted colored lines are the standard error for each time series. The K_G of all bursts are normalized into a range of $0 \sim 1$. The black dotted lines are fitted curves regressed by $K_G = c/(b + t^a)$.

By focusing the increasing phase of the bursts, about the endogenous bursts, the kinetic energy increased gradually. In contrast, about the exogenous bursts, the kinetic energy increased suddenly. However, by focusing the decreasing phase of the bursts, the differences between the endogenous and exogenous burst were smaller. We used the DTW measurement to compare the pre and post curves for the exogenous and endogenous bursts (Fig. 2.13). The result indicated that increasing phases of the endogenous and exogenous bursts are statistically distinct and that the difference remained valid after the hive entrance was opened to allow forager bees to forage although the DTW differences are bit smaller after the entrance was opened.

These result suggests that the mechanisms behind each type of burst are different. Exogenous bursts are like earthquakes that are caused by a stimulus from the outside of the colony. In contrast, Endogenous bursts can be caused by spontaneously. Also, the decreasing phase of the both types of the bursts was not so different. This result suggests that there is no difference between the mechanism of both types of bursts. We speculate the decay of bursts result from the energy consumption of individuals.
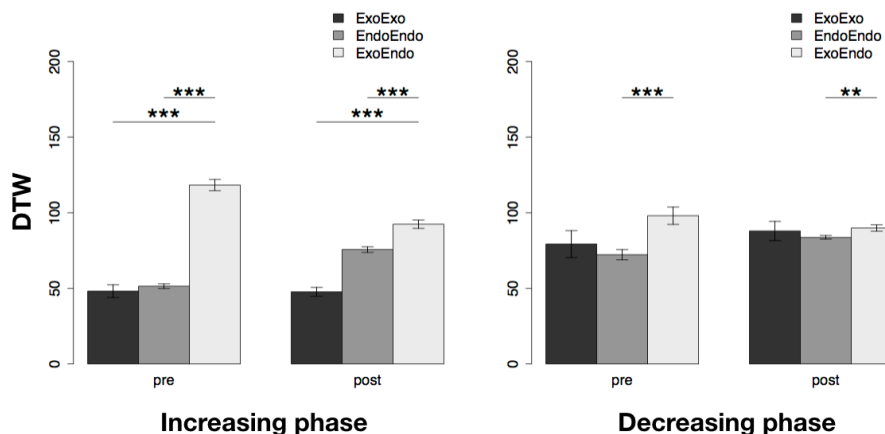
Figure 2.13: Statistical analysis comparing the increasing/decreasing phase of all trials by DTW distance. Left: the increasing phase. Right: the decreasing phase. "Pre" refers to before the hive entrance opens, and "post" refers to after the hive entrance opens. EndoEndo compares endogenous bursts; EndoExo compares endogenous and exogenous bursts; ExoExo compares exogenous bursts. The p-value was determined by Steel-Dwass test. $** < 0.01$, $*** < 0.01$

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2.3.3   What is the cause of burst propagation?

In the previous section, two types of bursts were observed in the honey
bee colony. Exogenous bursts are like earthquakes that are caused by a
stimulus from the outside of the colony. Endogenous bursts can be caused
by spontaneously.

In exogenous bursts, bees in the hive can be co-activated at a time which
causes a global burst. What causes the endogenous burst?

As mentioned in the introduction, it is well known that burst synchro-
nization is also observed in neurons. The action potential(spike) of neurons
spread throughout the network via the synapse connection. Bursts in social
network service(like Instagram, Twitter and so one) are also known to cause
bursty phenomena as some people's remark spreads throughout the commu-
nity via word of mouth[51][52][53][54]. In the honeybee's group, what is the
medium for the endogenous burst?

We assume the hypothesis that the hive will get activated by continuing
the chain reaction that the inactive bee becomes active by touching the active
bee.

First, we divided(binarize) K_i into the active state and the inactive state.
We used half of the average K_i as the threshold dividing the active and inac-
tive states of the workers. On the time series of binary K_i of a certain inactive
state, we counted how many active bees will come close to the inactive bees
within a certain time window τ. Then, we calculated the probability as a
function of how many times inactive bees are hit by active bees. We set $\tau =
60$ (sec).
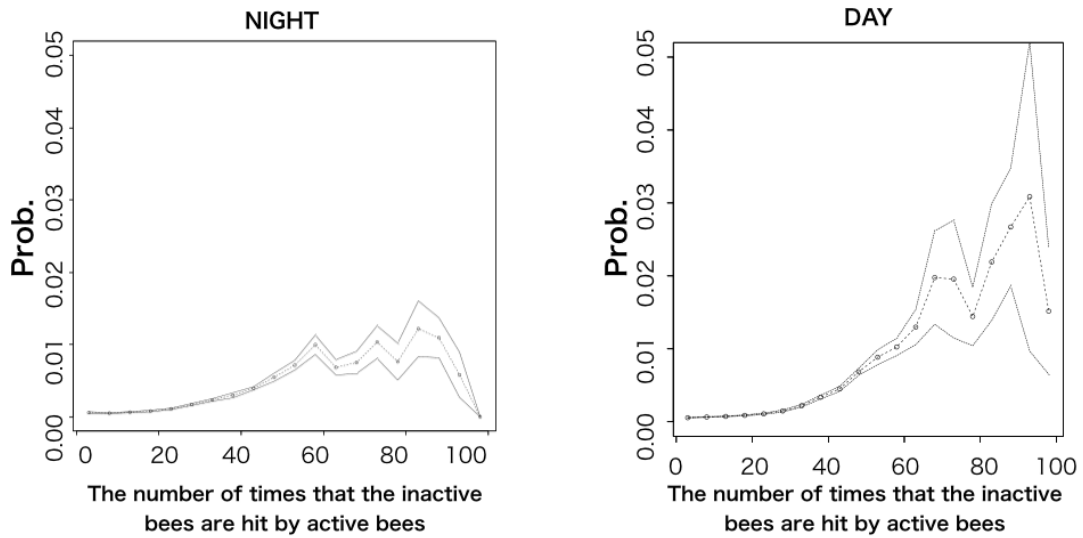
Figure 2.14: Histogram of the number of the times that inactive bees were hit by active bees and the inactive bees changed their state to active. Dotted lines describe the average and the solid lines describe the standard error. Left: Night time(8PM to 4AM) Right: Day time(4AM to 8PM).

As in Fig. 2.14, the probability that the bees in the inactive state changes to the active state increases as it comes in contact with the bee of the active state. Because bees are a diurnal insects, the probability at the night time seems to be lower than that of the day time. If the bee in the inactive state changes to the active state regardless of contact with the bee of the active state, the probability should be flat. This result suggests that inactive bees become the active state when they are in contact with active bees.

2.4 Discussion

We explored the endogenous and exogenous burst activity of beehives. First, we introduced a measure of global kinetic energy K_G to quantify the collective behavior of the hives, which demonstrated bursting behaviors. These bursts were quantitatively classified as endogenous or exogenous based on their evolutions over time of $K_G(t)$. The features of exogenous bursts were sudden increases in $K_G(t)$, which occurred because of external stimuli. On the other hand, the features of endogenous bursts were gradual increases in $K_G(t)$, which occurred spontaneously, potentially resulting from intrinsic bee interactions (Fig. 2.12, Fig. 2.13).

Our results indicated that during exogenous bursts, the activity level of many bees increased at the same time as a result of an external stimulus, whereas the organization of the endogenous bursts was triggered by some intrinsic bee's activity and spread into the hive as a more gradual chain reaction (Fig. 2.14).

This bursting behaviors of honeybee hives have never been previously reported. However, a similar phenomenon has been reported in entirely different research fields. For example, analyses of the number of video views on YouTube[51] and book sales from Amazon[53] reported two distinct types of the bursts: exogenous bursts caused by outside advertisements and endogenous bursts caused by online reviews (i.e., word-of-mouth communication).

In the burst seen in Youtube, twitter, etc., the medium for creating that burst will be word-of-mouth communication or Retweet. In the honeybees' group, Figure 2.14 suggested the direct/physical contact is the one of the possibility of the medium for the burst.

Early studies on synchronization of circadian rhythms so far have concluded that direct contact is essential and volatile pheromones are not used to synchronize circadian rhythms. However, a recent study suggests that direct contact (for example, via contact pheromones or tactile communication) is not necessary for synchronization of circadian rhythm in the hive.[55] This suggests is not consistent with our evidence, but there is possibility that the mechanism of entrainment of the circadian rhythm and entrainment of the burst are different. Also, the recent study speculated that volatile pheromones, hive odors, vibrations or changes in the neighborhood's microenvironment (for example, CO_2 concentration and humidity) are effective for the entrainment. In our study, we could not track these factors, but primarily, vibrations and microenvironment from other individuals may also have a role in the medium for the burst. Also, the global burst probably changes the local microenvironment and the temperature in the hive even though the hive is tightly thermoregulated. By accumulating microscopic

changes in the nest's environment due to these bursts, it may affect the time structure of a larger cycle such as circadian rhythm. It seems to be necessary to establish a different experimental system as to how the circadian rhythm and burst are related.

Chapter 3

Agent based model inspired by real honeybees

3.1 Introduction

In the previous chapter, we analyzed the activity levels of the beehive and of the individual bee activity. First, we introduced K_G to quantify the collective synchronized behavior that demonstrated bursting behavior in a colony of honeybees. We classified as endogenous and exogenous. The features of exogenous bursts were sudden increases in $K_G(t)$, which is caused by external stimuli. The features of endogenous bursts were gradual increased in $K_G(t)$. It may be caused by the interaction among bees (Fig. 2.12).

The organization of the endogenous bursts was triggered by some special bees' activities and spread into the colony. Furthermore, Figure 2.14 suggests that the inactive bees become active by contact with active bees, and that the entire hive enters into the bursting state.

The problems to be addressed in this chapter to confirm the bursts are only by direct/close distance interaction of bees. Here, based on the above results, we composed a computational model inspired by SIR model (Epidemic model).

3.2 Model/Analysis

To investigate the mechanism of the bursting behavior of the honeybees, we created an agent based model inspired by SIR model. SIR Model describes an infectious disease spread in a population[56][57]. As in the following, the agent is either in Susceptible, Infected or Recovered state, and the state changes according to the following fomula:

$$\begin{cases} S + I \xrightarrow{\alpha} 2I \\ I \xrightarrow{\beta} R \\ R \xrightarrow{\gamma} S \end{cases} \tag{3.1}$$

where α, β and γ are the reaction rate which are the controllable parameters.

The total number of agents are fixed, so that S + I + R is preserved.

We use the SIR model to describe the dynamics of honeybees. Bees are in the inactive (I), active (A) and refractory (R) state.

$$\begin{cases} I + A \xrightarrow{\alpha} 2A \\ A \xrightarrow{\beta} R \\ R \xrightarrow{\gamma} I \end{cases} \tag{3.2}$$
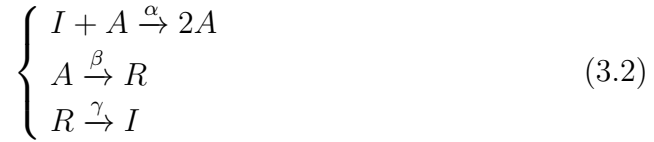
An inactive agent transits its state to the active agent with the probability α when the inactive agent contact with another active agents.

Active agents change to refractory agent with probability β spontaneously, and also refractory agents change their state to inactive state with probability γ. Here, refractory agent is an agent that has the same basic properties as the inactive state but does not change its state to the active state even if it contacts another active agents. The biological interpretation of the refractory state is that the bee can not become active state due to fatigue.

Also, I becomes A with a certain probability as

$$I \xrightarrow{\delta} A \tag{3.3}$$

To characterize the honeybee's motion and decide the agents' motion, we calculated the diffusive speed of the motion of the honeybee as:

$$E_{t_0}[(x(t + t_0) - x(t_0))^2] \propto t^\theta \tag{3.4}$$

where E_{t_0} represents the average with respect to the variable t_0. $x(t_0)$ is the site of a honeybee at time t. If the θ is greater than 1, it implies an anomalous diffusion. $\alpha = 1$ implies the Gaussian random walk. The average of $\theta = 1.2$.
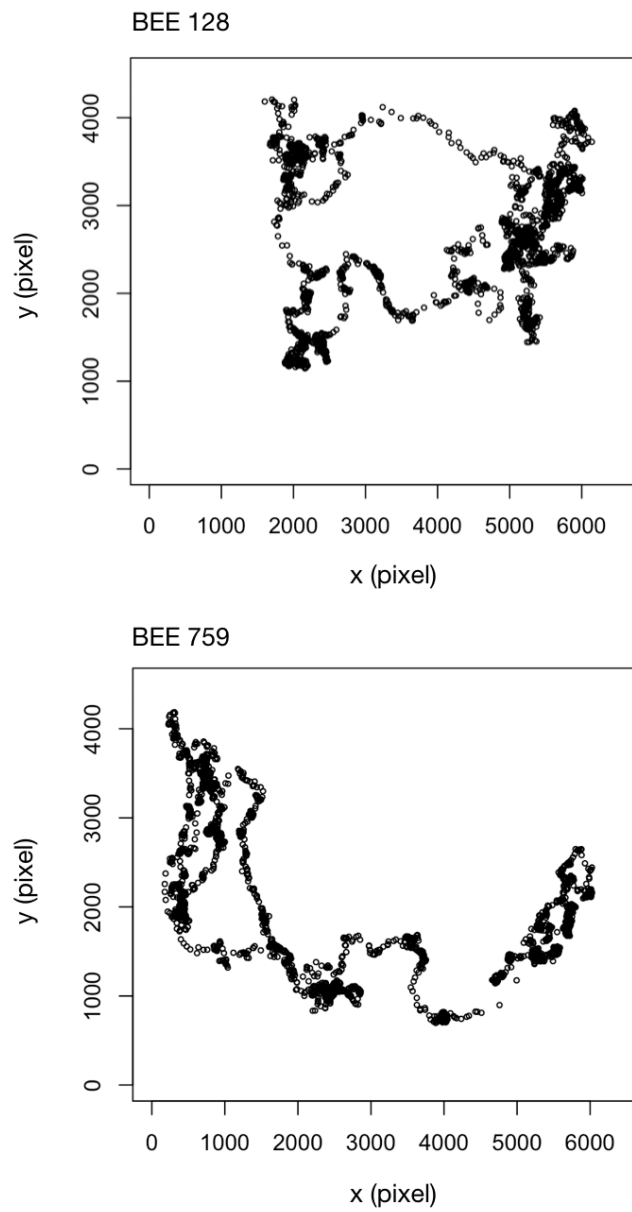
Figure 3.1: Example of the spatial trails of the honeybees in the hive. The duration is 3,600 sec.
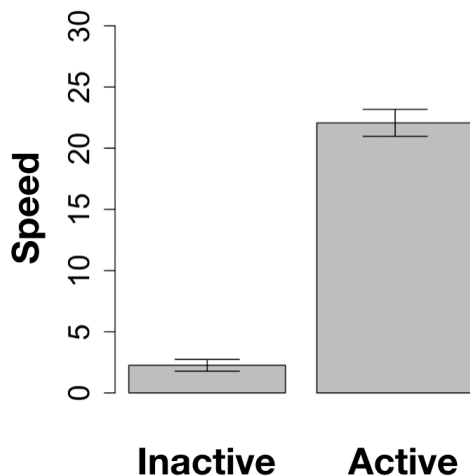
Figure 3.2: Mean speed of the inactive bees vs. the active bees. the error bar describes
the standard error. The mean speed of the active bees is 10.27 times larger than that of
the inactive bees.

We infer that the active/inactive states of the individuals are one of the
causes of the anomalous diffusion in their locomotion activities. The active
bee moves approximately ten times faster than the inactive bee(Fig. 3.2). In
this study, agent move by the gaussian distribution with $\mu = 0$ and $\sigma = 1$
and the active agent moves ten times faster than the inactive agent.

The form of the agent in the simulation space was a circle with a di-
ameter of five (pixels), and no difference was made between the head and
the abdomen. The occupancy density D of the agent in environment (hive)
was set to 0.4 which is the same with the actual experimental environmental
conditions (Fig. 3.3).

When the agent touches the boundary of the environment, it multiplies
the agent's speed by -1. That is, the environment is not a periodic boundary
condition. Also, when agent i comes in contact with agent j, both speeds
are multiplied by -1. The collision judgment was made when the distance d_{ij}
between agent i and agent j became 5. At the start of the simulation, agent
state and positions of the agents were initialized by the random values. In
the analysis, the simulation time of 1 trial was set to 50,000 steps, and the
initial 10,000 steps were removed. The results of the subsequent analysis are
the average of 10 trials.

3.3 Results

In the previous section, we analyzed the activity levels of the beehive and of
the individual bee activity. First, we introduced K_G to quantify the collective
synchronized behavior that demonstrated bursting behavior in a colony of
honeybees. We classified as endogenous and exogenous. The features of
exogenous bursts were sudden increases in $K_G(t)$, which is caused by external
stimuli. The features of endogenous bursts were gradual increased in $K_G(t)$.
It may be caused by the interaction among bees (Fig. 2.12).

The organization of the endogenous bursts was triggered by some special
bees' activities and spread into the colony. Furthermore, Figure 2.14 suggests
that the inactive bees become active by contact with active bees, and that
the entire hive enters into the bursting state.

The problems to be addressed in this section to confirm the burst are
caused by direct/close distance interaction of bees. Here, based on the above
results, we made a computational model inspired by SIR model (Epidemic
model).

Figure3.3.B showed the example of the simulated time series of global
activities K_g, however, needless to say, the time series pattern is dependent
on the parameters. Here we fixed these parameters.

First of all, we examined that the relations between the parameters (α,
β and γ) and the ratio between the mean K_g and the maximum value of the
K_g.

A
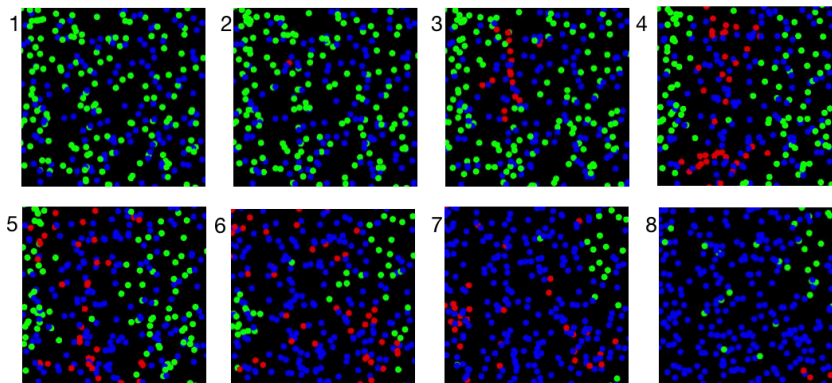
B
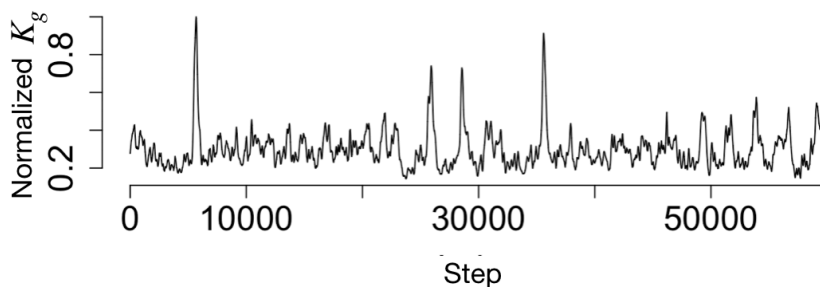
Figure 3.3: Examples of the simulation. (A) The snapshot of the simulated space and the agents. The time sequence is from the top-left to the bottom-right. (B) The example of the time series of K_g. α is 0.1, β is 0.025 and γ is 0.0001

3.3.1   Phase space of the parameters

Fig. 3.4 shows the relations between the parameters (α, β and γ) and the ratio between the mean K_g and the maximum value of K_g. When β smaller than 0.0, the ration is small. The active bee can not change the refractory bees. In addition, even if there is an agent in the inactive state, since the active agent is overflowing around, the inactive agent turns into active agent right away, so the hive was always bursty.

When γ was near to 0.00001, the agent can not come back from the refractory state, and when γ is near to 0.1, the agent suddenly change from the refractory state to the inactive state, and then it become to active, so

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the hive was always bursty.
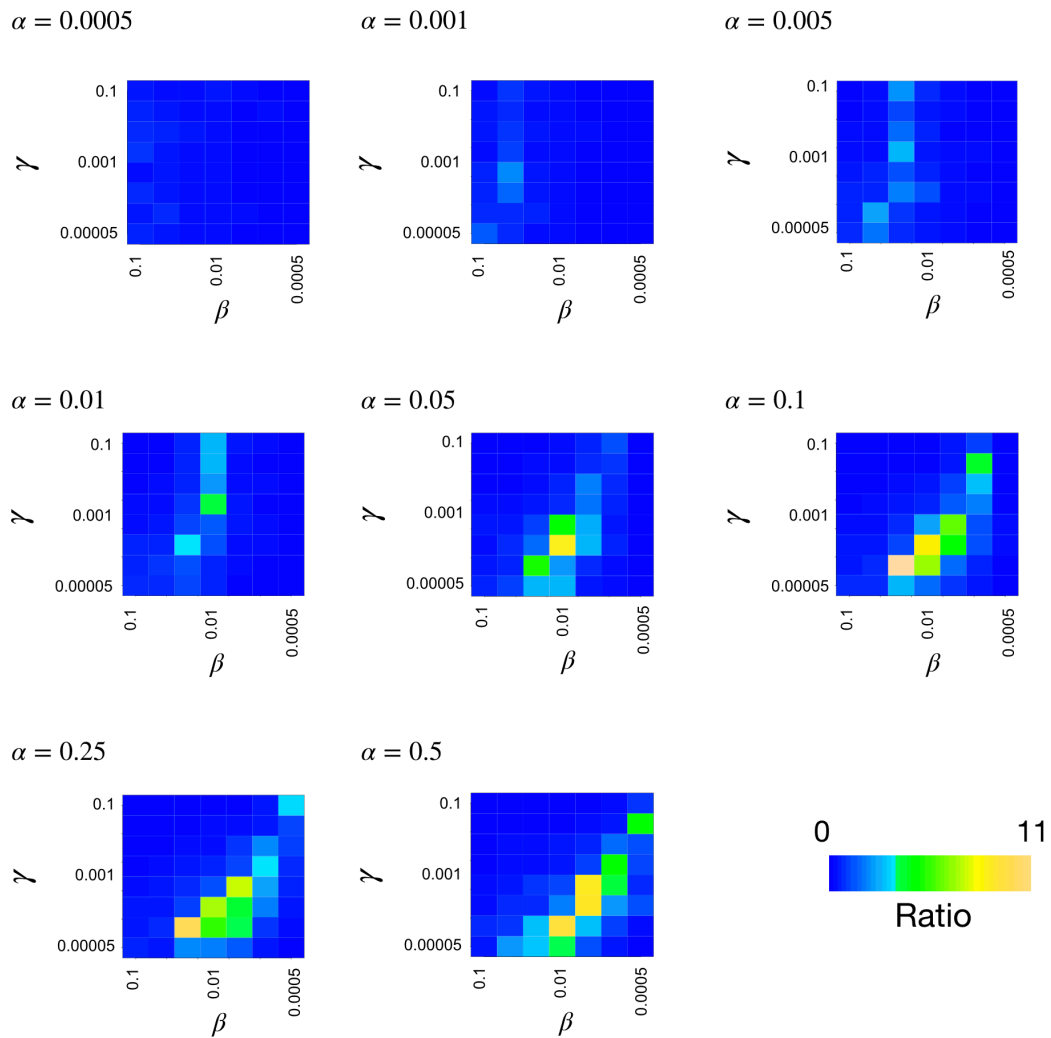
Figure 3.4: Phase space of α, β and γ. The colors describe the ratio between the mean K_g and the maximum value of the K_g.
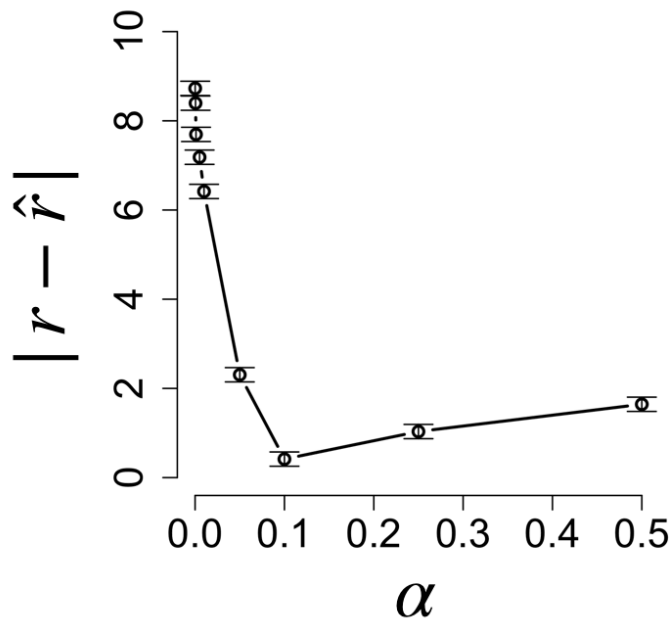
Figure 3.5: Relation between α and the similarity with the empirical data. r is maximum value of the ratio between the mean simulated K_g and the maximum value of the simulated K_g. $\hat{r}$ is the empirical ratio observed in the empirical data(10.29 ± 0.16).

For each alpha, the largest ratio was examined while changing the value of alpha. Fig. 3.5 shows that when α is 0.1, the ratio $\frac{max(K_g)}{mean(K_g)}$ is most similar to the empirical ratio. Here we fixed $\beta = 0.025$ and $\gamma = 0.0001$ because the ratio between the mean K_g and the maximum value of the K_g is most similar to the empirical result($\hat{r} = 10.29 \pm 0.16$).
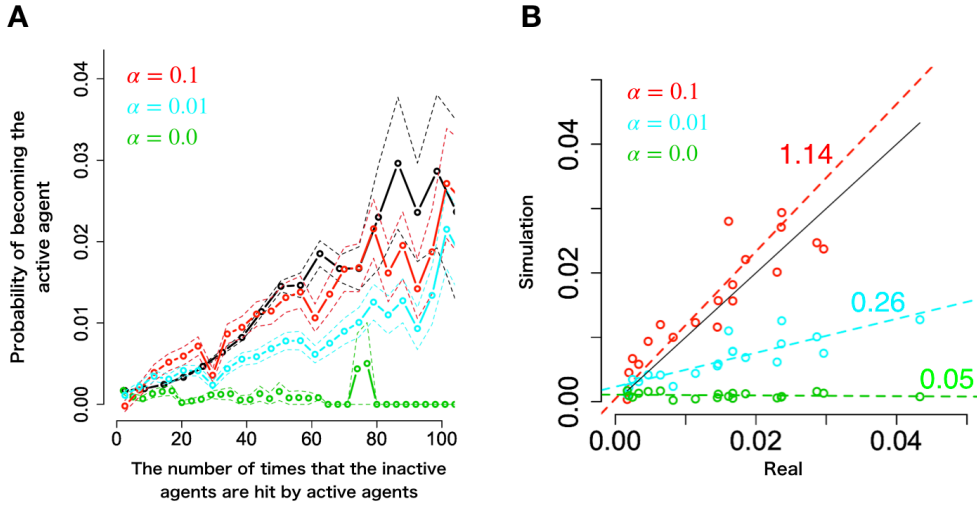
Figure 3.6: (A) The histogram of the number of the times that inactive agents were hit by active agents and the inactive agents changed their state to active. Black line describes the empirical data. Red line describes $\alpha = 0.1$, blue line describes $\alpha = 0.01$ and green line describes $\alpha = 0.0$. The other parameters were fixed to $\beta = 0.025$ and $\gamma = 0.0001$. The dotted lines describe the standard errors. (B)The correlations between the empirical data points vs. the simulated data points ($\alpha = 0.0$, 0.01 and 0.1) drawing in Fig 1.4.A. The colors description is the same with Fig 1.4.A. The black solid line describe $y = x$ and the dotted lines are the fitted lines by the liner regression. The values near to the fitted lines are the slopes of the fitted lines.

To reinforce these selected parameter values, we also calculated the probability as a function of how many times inactive bees are hit by active bees in the same fashion with the previous chapter. we set $\tau = 60$ (step)(Fig. 3.6.A). When α is 0, the probability is basically 0, because the agents become to active state spontaneously and no interaction is needed. On the other hands, when α larger than 0, the probability that the bees in the inactive state changes to the active state increases as it comes in contact with the bee of the active state. Also, the slope of the function is dependent on α. When alpha is 0.1, the slope of the function seems to be similar to the empirical data.

To measure similarity with experimental data in more detail, we used the scatter plot of the probability of becoming the active agent. In Figure 3.6.B, the result show that the slope of the scatter plot is the nearest to 1 whenα is 0.1 (If the simulated data and the experimental data is completely same, all data points are on the line of y = x.) Based on these estimation, in this study, we fixed α is 0.1.

Finally, we need to fix δ which is related to the frequency of the global

bursting behaviors (Fig.3.7). To fix δ, we measured the similarity of the
distribution of K_g and the distribution of the inter-burst-intervals between
the empirical data and the simulation. We used Jensen-Shannon divergence
to measure the similarity among the distributions (Fig.3.7.C). Based on these
estimation, in this study, we fixed δ is 5.0×10^{-5}.
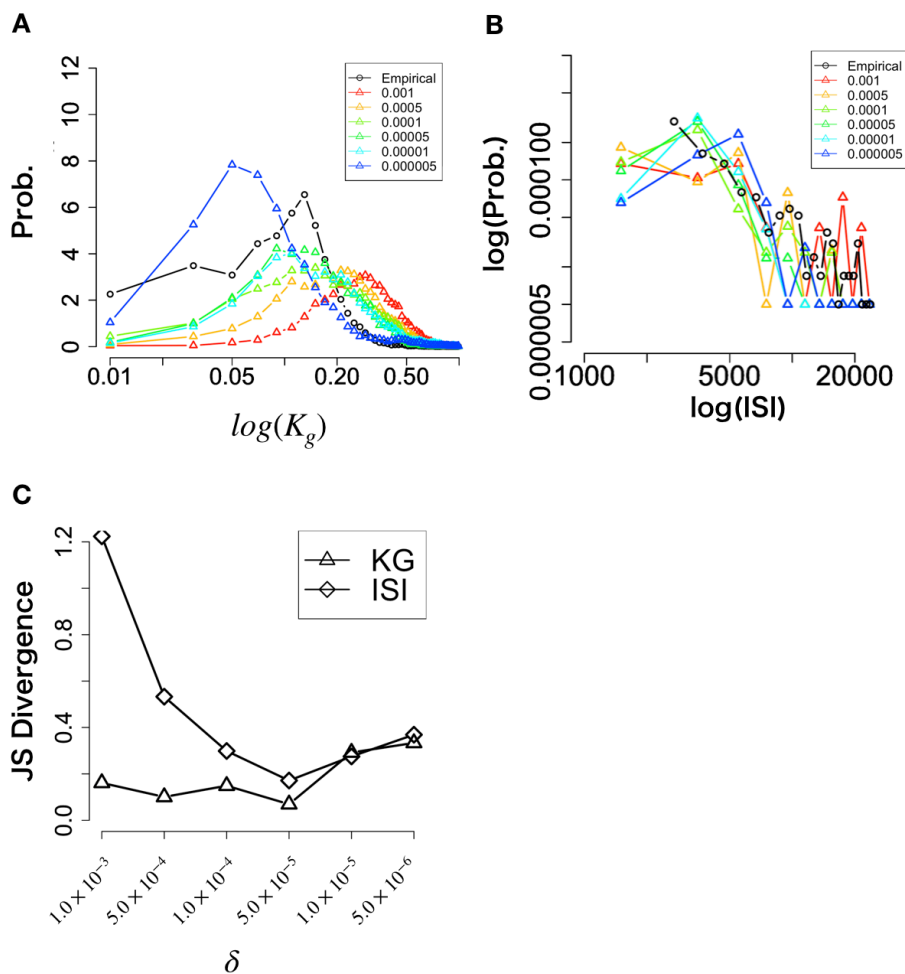
Figure 3.7: Parameter estimation for δ. δ is related to the temporal bursting patterns.(A)
The distributions of the K_g. The black line describe the real honeybees K_g. The colored
lines describe the distributions of the simulated K_g and each color means different δ.
(B) The distributions of the inter-burst interval. The black line describe the distribution
of the inter burst intervals of the real honeybees during daytimes. The colored lines
describe the distribution of the inter burst intervals of the simulation and each color means
different δ.(C) The Jensen-Shannon divergences between the empirical distribution and the
simulated distributions. The x-axis is δ and the y-axis is Jensen-Shannon divergence.

3.3.2 Comparison to the real honeybee

Based on the parameters selected in the last section, we characterize endogenous burst (i.e, the slope of the increasing phase) obtained from experimental data could be reproduced by the simulation. Furthermore, we discuss whether the probability α when the inactive agent turns into the active agent is homogenous, or there is variation among the individuals like trophallaxis network. The existence of the trophallaxis' s network implies that at least in m-to-m interaction between the bees is heterogenous.

Here, we simulated the bursting behavior of the honeybees under two different conditions. The first condition is "homogenous condition": α of every agent was the same value 0.1. The second condition is "heterogenous condition": α of each agent were different. To make the heterogenous condition, we used the degrees of the trophallaxis networks. We first calculated mean degrees of the trophallaxis networks of each individual through a trial and also did that for the every trial and constructed the list of these data. Then we normalized so that the average of the list is 0.1 and we randomly sampled a value from this normalized list and set it as α of each agent.
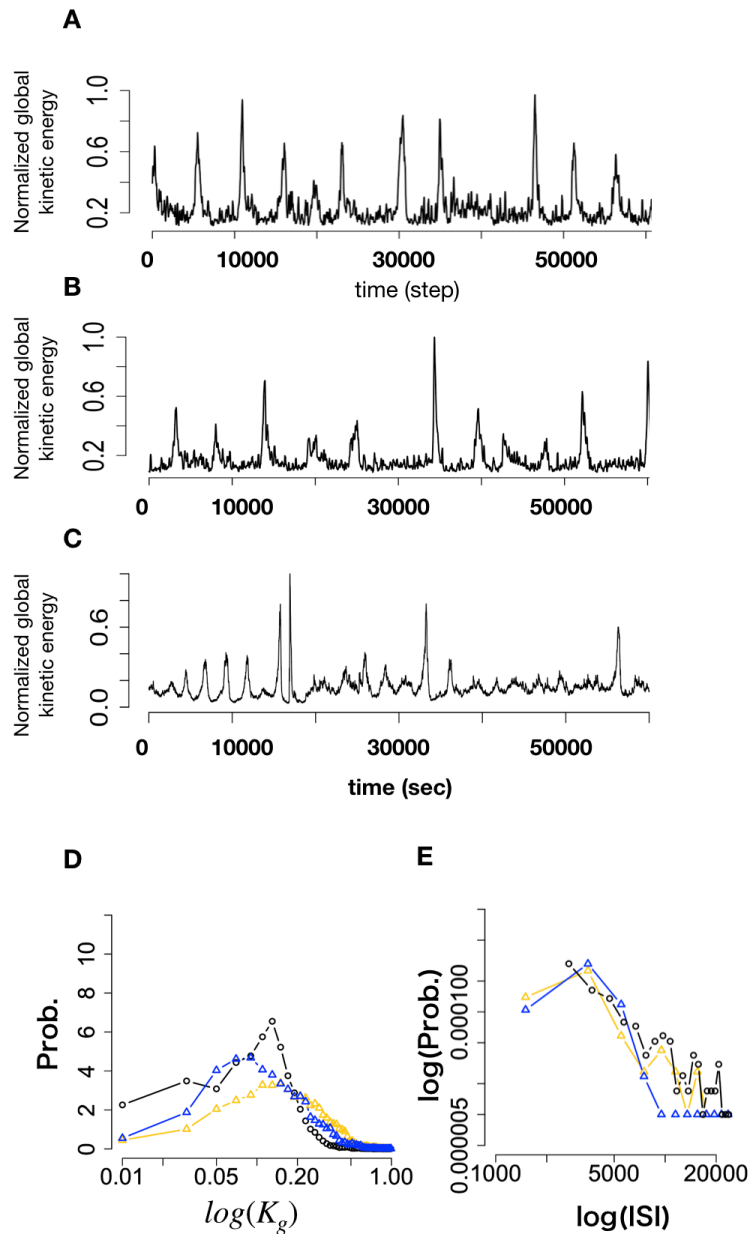
Figure 3.8: Examples of time series of K_g. (A) Simulated time series of K_g whose $\alpha = 0.1$("homogeneous condition"). (B) Simulated time series of K_g whose α obey to the same distribution with the real trophallaxis network("heterogenous condition").(C) The real time series of K_g of the honeybees(Trial 1201, pre-phase). (D) The distributions of the K_g. The black line describe the real honeybees K_g. The blue line describe ("heterogenous condition"). The yellow line describe ("homogenous condition"). (E) The distributions of the inter-burst interval. The black line describe the distribution of the inter burst intervals of the real honeybees during only daytimes. The blue line describe "heterogenous condition". The yellow line describe "homogenous condition".

Fig 3.8 shows that the simulated time series of K_g and the real time series of K_g are similar in qualitatively, however, the distributions of K_g are different from each other. The peak of the distribution of the real honeybees' K_g is smaller than that of the simulated K_g under the "homogeneous condition". Also, the peak of the distribution of the real honeybees' K_g and that of the simulated K_g under the "heterogeneous condition" are similar than that under the "homogeneous condition". (The Jensen-Shannon divergences between the empirical distribution and "homogeneous condition" is 0.25. The Jensen-Shannon divergences between the empirical distribution and "heterogenous condition" is 0.13). Figure 3.8.E shows that the inter burst intervals of the simulated time series are also more similar in the "heterogenous" conditions. The Jensen-Shannon divergences between the empirical distribution and "homogeneous condition" is 0.13. The Jensen-Shannon divergences between the empirical distribution and "heterogenous condition" is 0.06. These results suggest the heterogeneity of the reaction rate(α) is effective for the time evolution of the global bursting pattern.

On the other hand, the slopes of the increasing phase and the decreasing phase are almost the same despite of the different conditions and the value of the slope is same with the empirical data(Fig.3.9). This result suggests the heterogeneity of the reaction rate(α) is not effective for the mechanism of the increasing and the decreasing of the burst.

Also, Fig.3.9.A suggests that the behavior of the active bee are exponentially spread through the hive and the cause of the global bursting is the physical/close distance interaction of the bees. Fig.3.9.C, the result suggests that the real active bees spontaneously drop out from the global bursting behavior, because, in our SIR model, the active agents spontaneously drop out following the probability β.
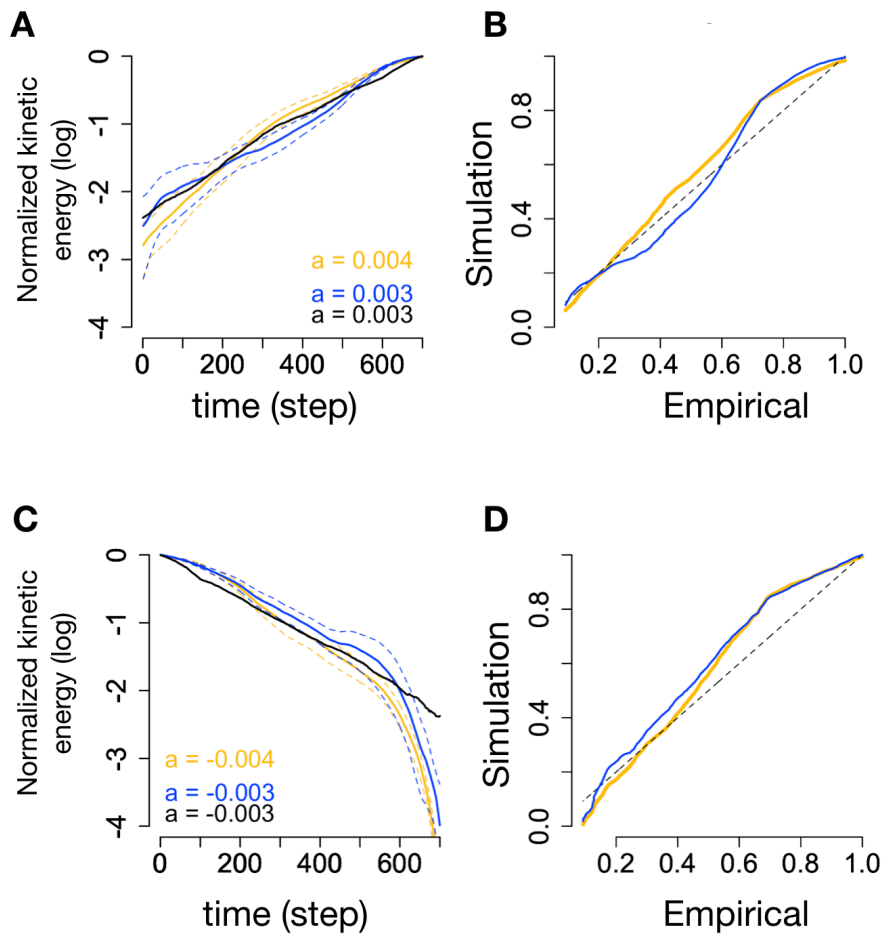
Figure 3.9: Comparison of the bursts between the empirical bursts and the simulated bursts. (A,C) The curves of the bursts. (A) Increasing phase. (C) Decreasing phase. Black solid line describe the average of all experimental trial. Yellow solid line describe the average of simulated data whose $\alpha = 0.1$("homogeneous condition"). Blue solid line describe the average of simulated data whose α obey to the same distribution with the real trophallaxis network("heterogenous condition").The all bursts were normalized into a range of $0 \sim 1$. The dotted lines are regression line fitted with the range of the x axis between $200 \sim 500$(increasing phase), $100 \sim 500$(decreasing phase). (B,D) The scatter plot between the curves of the empirical bursts and the simulated bursts. Yellow line describes the empirical curve vs. the "homogeneous" simulated curve. Blue line describes the empirical curve vs. the "heterogeneous " simulated curve. Black dotted line describes $y = x$. (B) Increasing phase. (D) Decreasing phase.

3.4   Discussion

As mentioned in the introduction, the question of what caused the social entrainment of bees has been investigated by various experiments. Especially in the study of synchronization of circadian rhythms, physical contact and trophallaxis were considered necessary in the early studies. However, in recent research, it is thought that the cause is not a physical contact but a small environmental change and vibration in the hive. On the other hand, in the burst of bees we discovered, the results of the previous chapter suggested the possibility that physical contact between the bees causes the burst.

The purpose in this chapter was to clarify whether physical/close distance interaction causes of burst, as in the simulation based on epidemic model. Figure 3.9 suggests that the mechanism behind the increasing phase of the endogenous burst is similar to the mechanism of our SIR model and the cause seems to be the physical/close distance interaction among bees. Moreover, the results about the decreasing phase of the bursts suggest that the real active bees spontaneously drop out from the global bursting behavior rather than the effect of the negative feedback. We speculate these "drop-out" are caused by the energy consumption of the honeybees.

The characteristics of the curves of the burst did not change when alpha was homogeneous($\alpha = 0.1$) or heterogeneous(α obey to the same distribution with the real trophallaxis' network and the average of the distribution $\mu = 0.1$) among individuals(log-normal like distribution), so we speculate that the heterogeneity of the reaction probability α is not efficient for the mechanism of the increasing and the decreasing of the burst. It is because the locomotion of agent obeys to a random walk and this randomness absorb and invalidate the heterogeneity.

On the other hand, about the more long-term time evolution of K_G (like the inter burst intervals), the heterogeneity seems to be effective (Fig. 3.8). Since the "heterogenous" condition's distribution of α obeys to log-normal like distribution, the α of the most agents are smaller than 0.1 and the α of the some agents are large under "heterogenous" condition. Therefore, the most agents' activities do not entrain each other but some "chosen" agents' activities spread to the hive.

There are many studies about SIR model which has the network structure. These studies suggest that the characteristics of the network structure are very important for the information spreading[58][59][60]. However at least the increasing and the decreasing of the burst, it is thought that the spreading of the K_i results from random interactions rather than the structured network.

To summarize the results of the simulation, our findings suggest that

physical/close distance interactions among bees cause of bursts, and once someone has triggered the burst, the subsequent time evolution of burst will proceed independently of their "individuality". On the other hand, the "individuality" is effective for the more long-term time evolution of their activities, which implies someone who triggers the burst may have specific characteristics.

Chapter 4

Classifying pioneer bees

4.1 Introduction

What is suggested by the results in the previous chapter is that the active state bees before an endogenous burst may trigger the global endogenous burst.

An important issue in collective behavior is how the group structure is influenced by individual personalities, such as how they influence the flow of information or how the decisions of specific individuals are given greater weight within the group[61], [62]. Previous studies have shown that leadership is related to various phenomena, such as spatial position within the group, position in the group 's affiliation network, etc[63], [64].

This chapter discuss the identification of the bees are initializing and committing to organize the global burst and their characteristics.

4.2 Methods/Analysis

4.2.1 Classifying bees ' behaviors by their activities and activity timings

To analyze when and which bees are initializing and committing to organize the global burst, we used non-negative matrix factorization (NMF), which has become a popular decomposition algorithm[66].

Given a non-negative matrix $\boldsymbol{X}$ ($m \times n$ matrix $\boldsymbol{X}$), this algorithm finds the non-negative matrix factors $\boldsymbol{W}$ and $\boldsymbol{H}$ such as:

$$\boldsymbol{X} \simeq \boldsymbol{W}\boldsymbol{H},$$

where $\boldsymbol{W} \in \mathbb{R}^{m \times r}$ and $\boldsymbol{H} \in \mathbb{R}^{r \times n}$ and r is the number of components (called *rank*). In practice, r is often chosen such that $r \ll min(m, n)$.

A critical parameter in NMF is the factorization rank r, which defines the number of representative bee activities used to approximate the target matrix. The way of setting the value of r is to try different values and compute some quality measures, and then choose the best value for r respectively. There are several approaches to decide the optimal value of r; for example, Brunet *et al.* proposed choosing the first value of r for which the cophenetic coefficient starts decreasing[67]. Hutchins*et al.* suggested choosing the first value where the residual sum of the squares curve presents an inflection point[68] . We chose to use Brunet's approach in this study. The advantage of this algorithm is that it can factorize the input matrix without destroying the cluster structure of the original matrix. To estimate r, we calculated the cophenetic coefficient with changing r from two to ten.
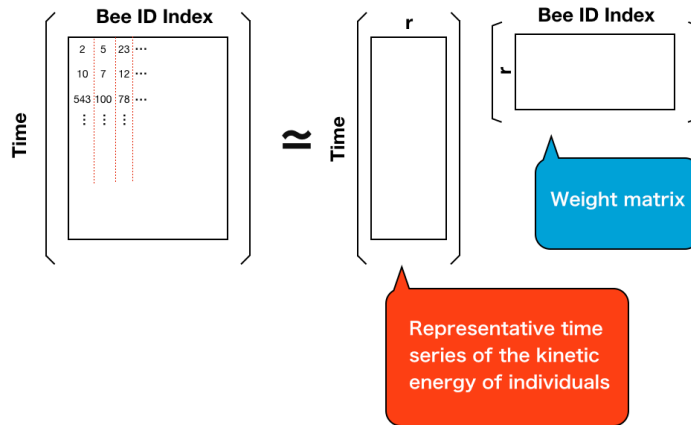
Figure 4.1: Description of NMF. The left matrix is the original matrix which contents are the time series of K_i of every bee. The middle matrix is called "base matrix" which contents are the representative time series of K_i. The right matrix is called "weight matrix". r defines the number of representative bee activities used to approximate the target matrix.

In general, $\boldsymbol{H}$ is called base(feature) matrix and $\boldsymbol{W}$ is called weight matrix.

In the case of our analysis, $\boldsymbol{X} \in \mathbb{R}^{m \times n}$ means the K_i matrix at the bursting phase determined by Kleinberg algorithm. n is the number of individual bees, m is the time length of the bursting-phase and the matrix elements are K_i. To extract $\boldsymbol{X}$ from the original K_i matrix, we examined the time stamps of the peak points of the each burst ts_b, where b is the index of the detected

bursts. Then we defined $\boldsymbol{X}_b$ as follow (Fig. 4.1):

$$\boldsymbol{X}_b = \begin{bmatrix} k_{ts_b-1000,0} & \cdots & k_{ts_b-1000,n} \\ \vdots & \ddots & \vdots \\ k_{ts_b+1000,0} & \cdots & k_{ts_b+1000,n} \end{bmatrix} \tag{4.1}$$

Note that we confirmed that the minimum interval of the peaks of the detected bursts is 2,538 sec (Fig. 2.8).

Also, $\boldsymbol{H}$ describes the representative examples of the time series of the K_i (the number of the example(base) depend on r).

The method starts from randomly initializing matrices W and H, which are iteratively updated to minimize a divergence functional. To estimate matrices W and H as a local minimum, NMF algorithm solve the following minimization problem:

$$\min_{W,H \geq 0} [D(X, WH)] \tag{4.2}$$

where D is a loss function which based on the Kullback-Leibler divergence.

$$D : A, B \mapsto KL(A\|B) = \sum_{i,j} a_{ij} \log \frac{a_{ij}}{b_{ij}} - a_{ij} + b_{ij} \tag{4.3}$$

There are some type of update functions to solve the above minimization problem iteratively. In this study, we used "Multiplicative update rules" and repeated the following update functions:

$$\begin{aligned} H_{kj} &\leftarrow H_{kj} \frac{\left(\sum_l \frac{W_{lk} X_{lj}}{(WH)_{lj}} \right)}{\sum_l W_{lk}} \\ W_{ik} &\leftarrow W_{ik} \frac{\sum_l [H_{kl} X_{il}/(WH)_{il}]}{\sum_l H_{kl}} \end{aligned} \tag{4.4}$$

The initial entries of W and H are drawn from a uniform distribution. The number of iteration of the update function was 500. We executed 50 times with changing the initial matrices and then we chose the solution of which cost function is minimized.

4.2.2 Classifying "pioneer bee" using NMF

NMF decomposes the original matrix into a matrix composed of feature vectors and its weight matrix, so it is often used as a soft clustering algorithm.

The cluster member is computed as the index of the dominant basis component for each bee. For example, if the maximum value of the weight vector $\boldsymbol{w}(=\boldsymbol{W}_{(,beeZ)})$ of Bee Z is $\boldsymbol{w}_{i(1\leqq i\leqq r)}$, Bee Z is classified as ith base.

We determined that a "pioneer bee" is an individual corresponding to a base vector whose largest amplitude was observed before that of the global burst. Note that if the largest value of the recomposed K_i of Bee Z at Burst i is smaller than the mean $\boldsymbol{X}_i$, we did not identify Bee Z as "pioneer bee".

Such bees were identified for each identified burst. We created "Pioneer bee binary matrix" $\boldsymbol{PM}_{i,j}$ where the columns represent each burst index and rows represent an individual bee index. The matrix element was either 0 or 1: if a bee i was categorized as "pioneer bee" of the burst j, then the matrix element (i,j) was assigned 1; otherwise, it was 0.

4.2.3   Dimension reduction of the "Pioneer bee binary matrix"

To investigate the similarity of the combination of the pioneer bees in different bursts, we performed a dimensional reduction of $\boldsymbol{PM}_{i,j}$ using the non-metric multidimensional scaling (nMDS). A non-Metric Multi-Dimensional Scaling (nMDS) method is one of the dimensional reduction scheme that attempts to represent data points in a reduced dimension space where the distances between the points are preserved from the original data set[69]. The difference between the nMDS and MDS is that the nMDS consider the non-prametric monotonic relationship between the pair-wise distances in the projected MDS space and the distances in the original data dimensions.

Input data of nMDS is a distance matrix. In this study, at first, we created the distance matrix from "pioneer bee matrix". We used the Jaccard index for computing the distance of a binary matrix:

$$J(A,B) = \frac{|A \cap B|}{|A \cup B|} \tag{4.5}$$

where A is a group of pioneer bees in Burst A and B is a group of pioneer bees in Burst B. Then, we reduce the dimension of the distance matrix using nMDS. In the reduced space, the distance between the points shows the behavioral similarity of the pioneer bees in each burst. Therefore, if the same bees were pioneer bees during the different bursts, then these bursts are represented close to each other; otherwise, they were presented further apart.

The lost function of nMDS (known in the literature as "Stress") as follow:

$$STRESS = \frac{\sum \sum_{i \neq j} \left[\theta(d_{ij}) - \hat{d}_{ij} \right]^2}{\sum \sum_{i \neq j} \hat{d}_{ij}^2} \qquad (4.6)$$

where $d_{i,j} = 1 - J(i,j)$, $\hat{d}_{i,j}$ is distances in the projected low-dimensional euclidean space(here, the dimension is 2). θ is a monotonic function. In general, to solve this minimization problem, nMDS uses isotonic regression[70]. We iterated until the stress is smaller than 0.2.

4.3 Results

4.3.1 Classifying pioneer bees

We identified bees whose activity increased in the first half of a bursting phase as "pioneer bees" and we searched for common characteristics among them.
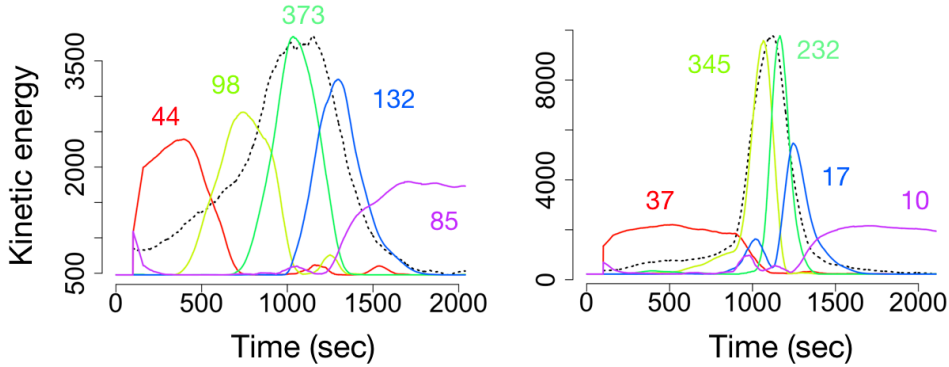
Figure 4.2: Examples of the result of NMF. The black lines describe global kinetic energy K_G. The colored lines describe the decomposed bases. (Left) Burst9 (Endogenous burst) were decomposed into 5 bases. (Right) Burst23 (Exogenous burst) were decomposed into 5 bases. The numbers describe the number of the categorized bees into each base.

To identify the pioneer bees, we applied NMF as described in the Methods section. Specifically, we factorized the matrix of the kinetic energy of individuals $K_i(t)$ in the vicinity of each burst to extract important individuals (the base matrix) and its weight matrix by NMF. Fig. 4.2 shows the difference between the endogenous (left) and exogenous (right) bursts by taking their averaged kinetic energy as the vertical axes. Two visible "modes"

emerged before the main peak of the endogenous, but there is only one in the exogenous burst. Counting the number of pioneer bees in each burst also revealed that there were more pioneer bees in the endogenous bursts than in exogenous ones (Fig. 4.3).
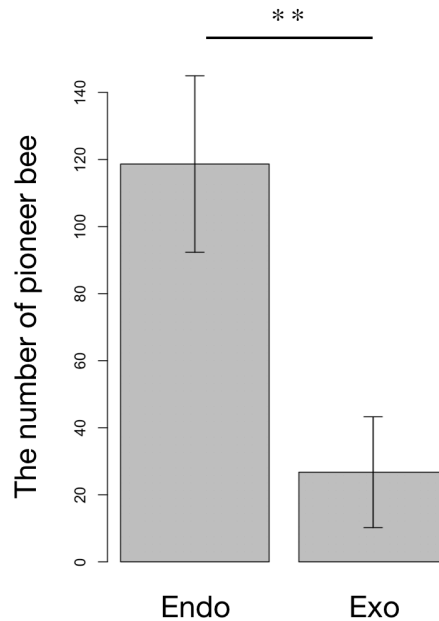
Figure 4.3: The number of pioneer bees in the endogenous and exogenous bursts in every trial. The p-value was determined by Brunner-Munzel test. $** < 0.05$.

Are pioneer bees selected randomly for each burst or is there any rule to select the pioneer bees?

The group of bees that had a high likelihood of becoming pioneer bees in the first half of the Trial 1201, less likely became pioneer bees in the latter half of Trial 1201(Fig.4.4). This result suggests that pioneer bee is not always pioneer bee, nor selected randomly. The role as pioneer bees seem to change as the time passes.
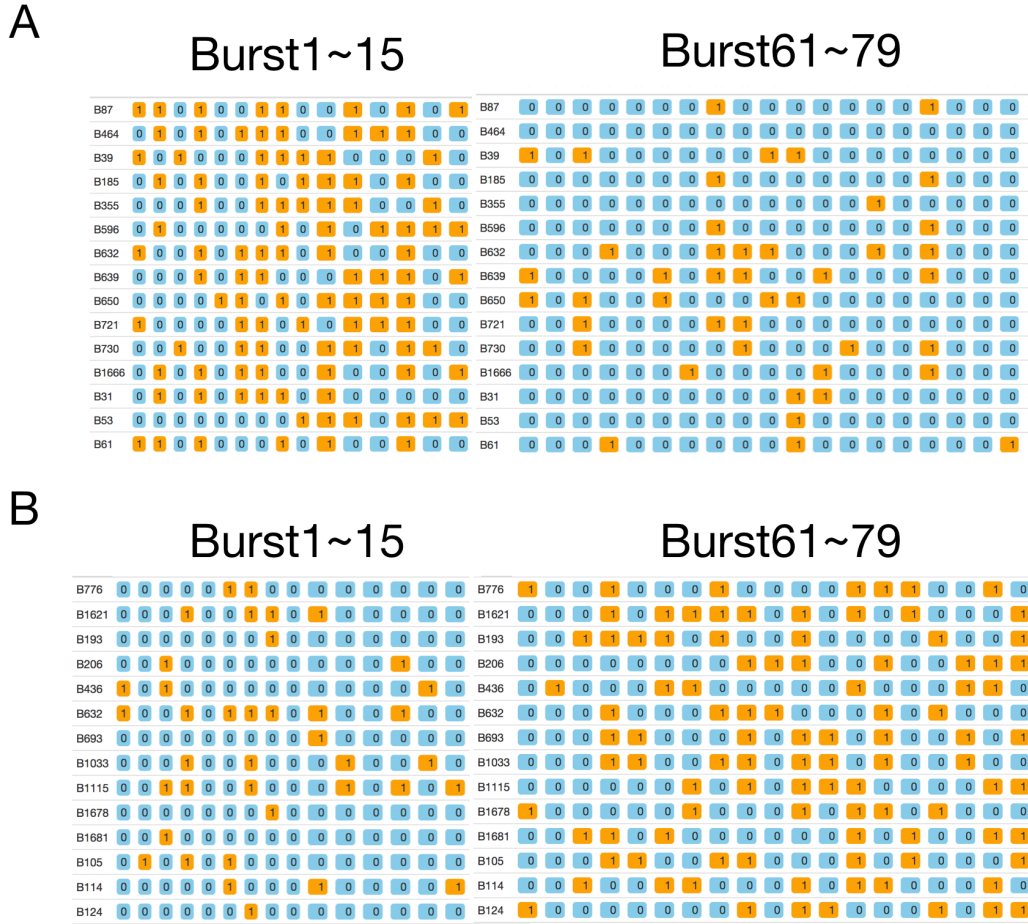
Figure 4.4: Example of pioneer bee matrix. The rows describe bee's indexes. The columns describe burst indexes. The yellow contents describe pioneer bees. (A) Bees' indexes are sorted by the number of times they become pioneer bees from Burst1 to Burst15. (B) Bees' indexes are sorted by the number of times they become pioneer bees from Burst61 to Burst79.

We further investigated the similarity of the combination of the pioneer bees in different bursts using the matrix $PM_{i,j}$ described in the Methods section. Figure 4.5 shows the results of the dimensional reduction of the $PM_{i,j}$ of Trial 1201 using multidimensional scaling (MDS). In this two-dimensional space, the distance between the points shows the behavioral similarity of the pioneer bees in each burst. Therefore, if the same bees were pioneer bees during different bursts, then these bursts were plotted near to each other; otherwise, they were plotted further apart.
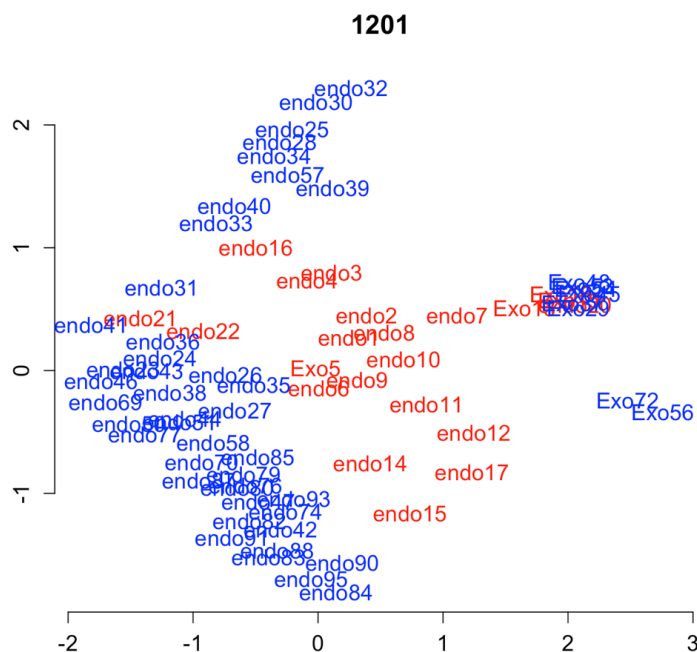
Figure 4.5: Classifying the bursts according to individual pioneer bees using MDS(Trial 1201). Bursts depicted in red occurred before the entrance of the hive was opened and bursts depicted in blue occurred after the entrance of the hive was opened. Also, the endogenous burst describes as "endo" and the exogenous burst describes as "Exo".

Interestingly, the bursts occurred before the entrance of the hive was opened (pre-phase) and the bursts occurred after the entrance of the hive was opened (post-phase) constitute clusters in the MDS space. This result suggests that the pioneer bees group at pre-phase and the pioneer bees group at post-phase are different. Also, focused on the post-phase, the bursts aggregated on the left-bottom of the figure correspond with the bursts in the daytime.

The exogenous bursts tend to aggregate or be plotted the different place from the the cluster of the endogenous bursts. The number of the pioneer bees of the exogenous bursts are smaller than that of the endogenous bursts (Fig.4.3) so that the elements of the pioneer bee matrix of the exogenous bursts are almost 0. Therefore the Jaccard distances between each exogenous burst are small. Thus, the exogenous bursts tend to aggregate on the same place of the nMDS space. These results were fundamentally observed in other trials.
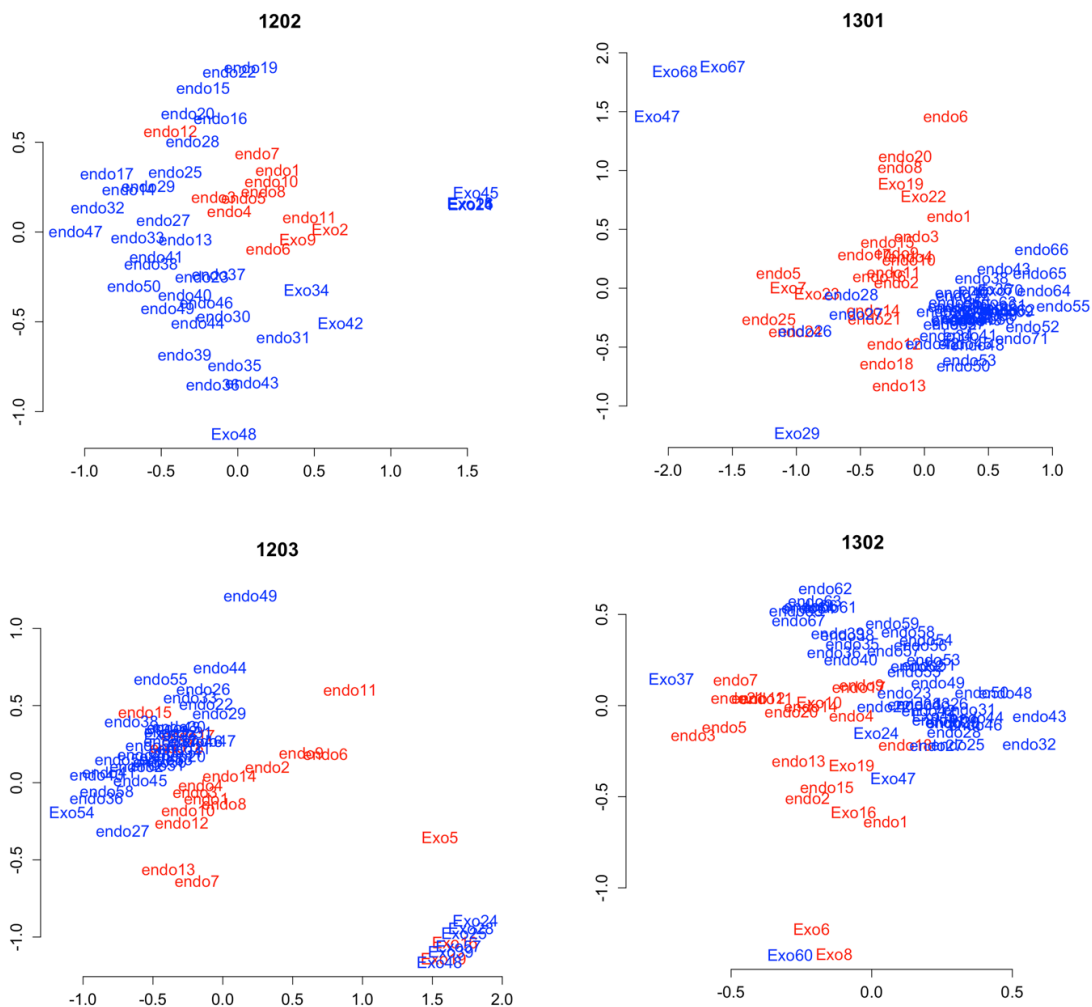
Figure 4.6: Classifying endogenous bursts according to individual pioneer bees using nMDS of the other trials. Bursts depicted in red occurred before the entrance of the hive was opened;. bursts depicted in blue occurred after the entrance of the hive was opened. Also, the endogenous burst describes as "endo" and the exogenous burst describes as "Exo".

We speculate that these bursts are related to daytime activities, including foraging behavior. We analyzed the relationship between forager and pioneer bees and found that, after the hive entrance was opened, more than half of the pioneer bees became forager bees. These results are shown in Figure 4.7 and Figure 4.8. Note that as we described in Methods, the definition of forager in this study is that the bee become absent continuously at least

more than half an hour, so the forager at pre-phase means that there are the bees which became the forager after the entrance was opened. In addition, the probability $forager \wedge pioneer/forager = 0.91$, which means almost all of the forager experiences the role of "pioneer bee." Note that the result of Trial 1202 is different from the other trials. We will discuss this result in Discussion chapter.
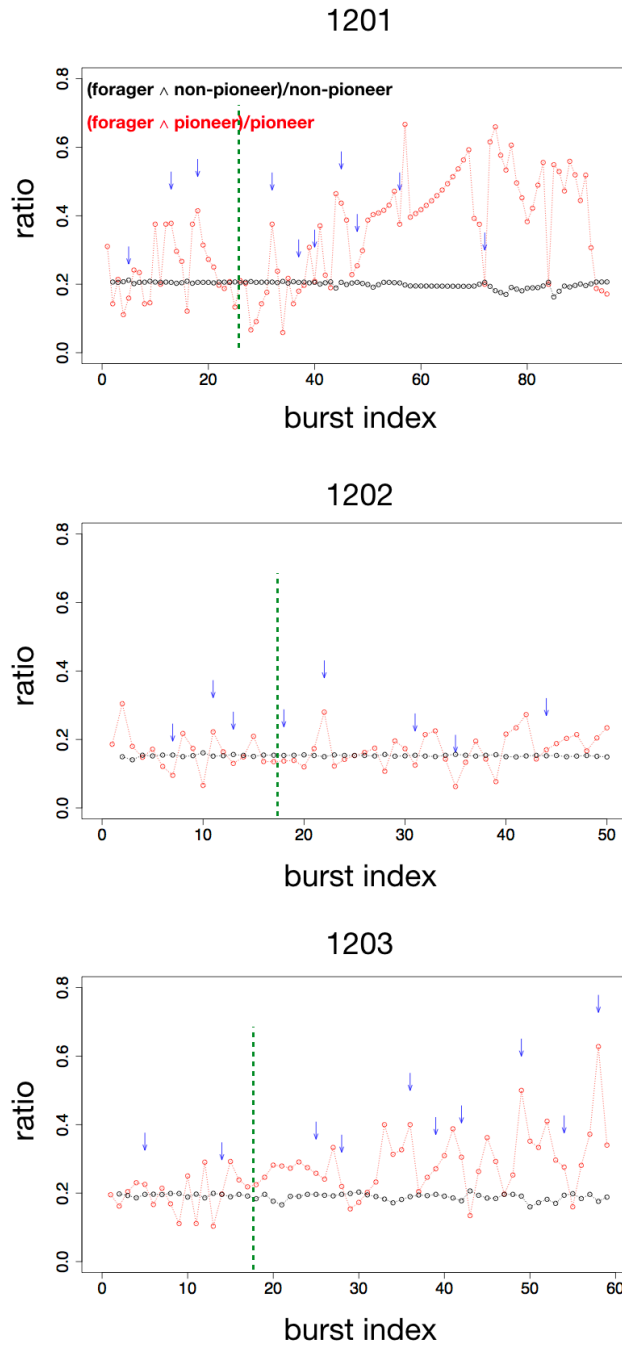
Figure 4.7: Ratios of foragers in "pioneer bees" and "non-pioneer bees" for Trial 1201,1202 and 1203. The time when the entrance was opened is depicted by the green dotted line.The exogenous bursts are depicted by the blue arrows.
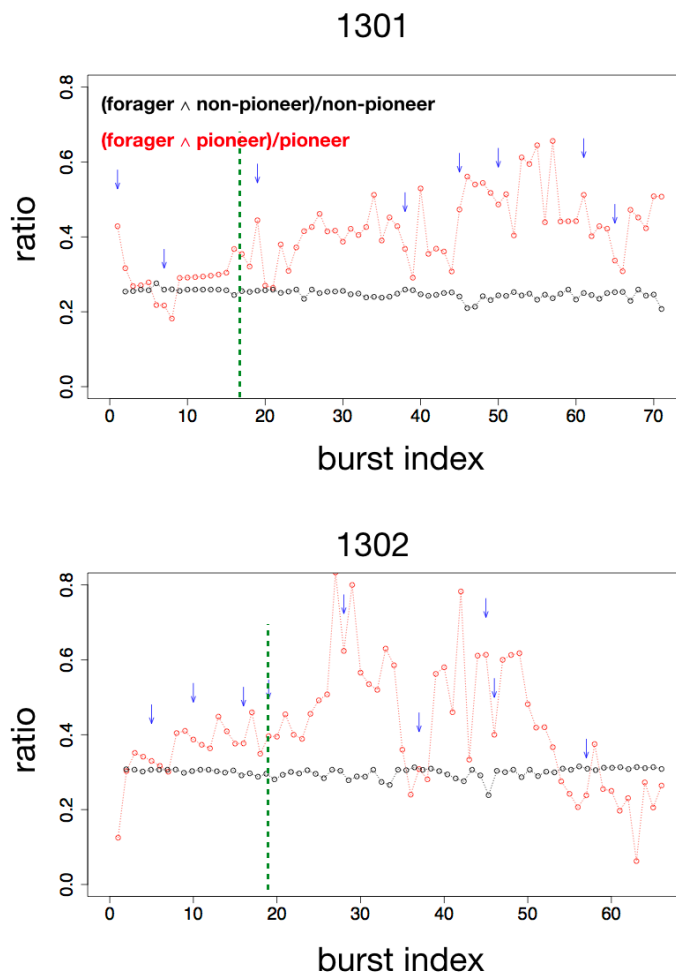
Figure 4.8: Ratios of foragers in "pioneer bees" and "non-pioneer bees" for Trial 1301 and 1302. The time when the entrance was opened is depicted by the green dotted line.The exogenous bursts are depicted by the blue arrows.

In addition, we analyzed the relationship between the trophallaxis and pioneer bee (Fig. 4.9). Pioneer bees tend to spend more time to the trophallaxis behavior than non-pioneer bees after the entrance was opened. However, before the entrance was opened, there is no significant difference of the mean frequency of trophallaxis between non-pioneer and pioneer. This result suggests that the pioneer bees have a role in the spreading of the information of the outside since some researches of the trophallaxis suggest the trophallaxis is related to the spreading of the information in the hive[71][72][43].
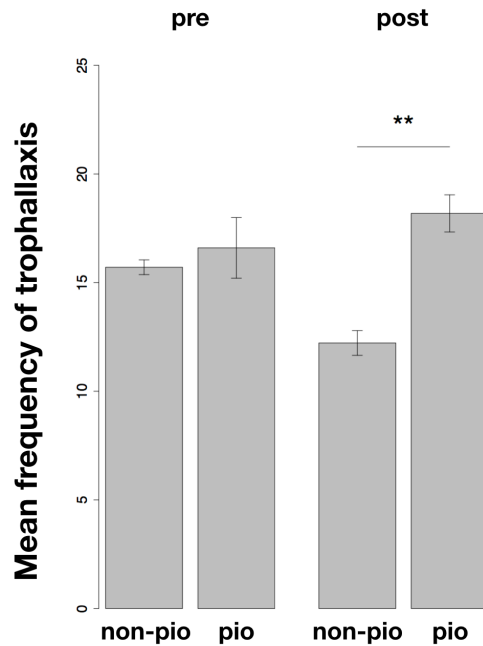
Figure 4.9: Pioneer bees tend to spend more time to trophallaxis than non-pioneer bees after the entrance was opened. "pre" means the period before the entrance was opened and "post" means the period after the entrance was opened. The p-value was determined by Brunner-Munzel test. $**<0.05$.

Moreover, we analyzed the relationship between the waggle dance and pioneer bee (Fig. 4.10). Pioneer bees tend to spend more time to the waggle dance behavior than non-pioneer bees. Since waggle dancer belongs to forager, this result is consistent that more than half of the pioneer bees were forager bees. Moreover, even though in the forager group, this tendency is same, means the foragers which did not perform the waggle dance do not become the pioneer bees. This result reinforces that the pioneer bees have a role in the spreading the information of the outside of the hive.
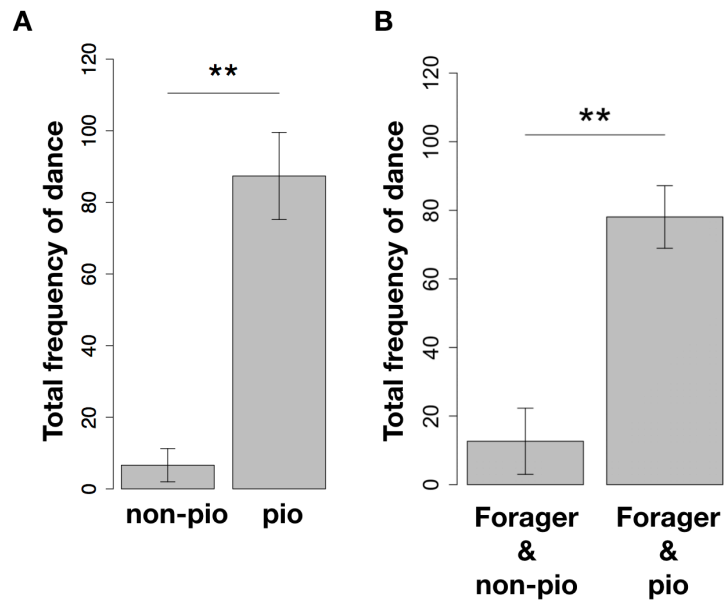
Figure 4.10: Waggle dance vs. Pioneer. (A) Pioneer bees tend to spend more time to waggle dance than non-pioneer bees. (B) Pioneer and forager bees tend to spend more time to waggle dance than non-pioneer and forager bees. The p-value was determined by Brunner-Munzel test. $** < 0.05$.

4.3.2 Spatial distributions of pioneer bees

Here, we investigated the spatial distribution of pioneer bees in the hive for each burst (Fig 4.11). At pre-phase, we could not detect the positive difference of the spatial distribution of pioneer bees. At post-phase, the pioneer bees tended to exist near to the hive entrance. This result is consistent with the result that more than half of the pioneer bees are belongs to foragers.
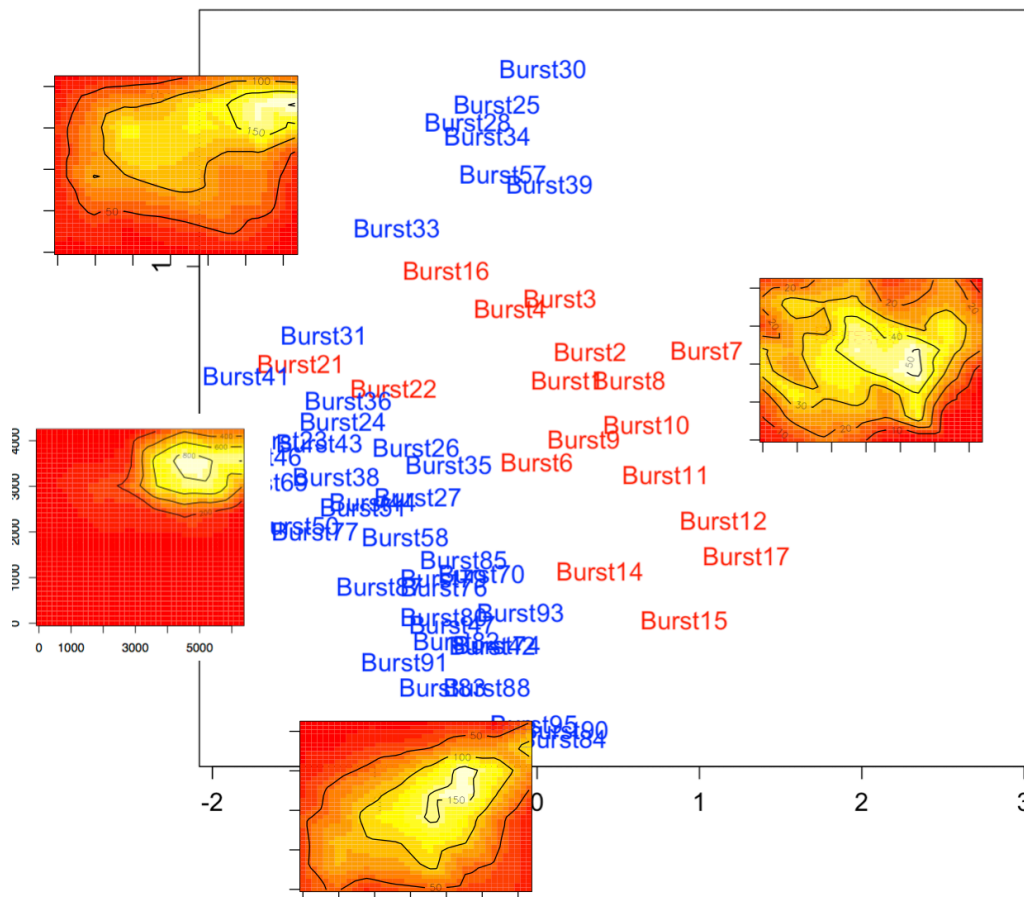
Figure 4.11: MDS space of Trial1201 and the spacial occupation pattern of the pioneer bees in the hive. Bursts depicted in red occurred before the entrance of the hive was opened;. bursts depicted in blue occurred after the entrance of the hive was opened. The right heat map describes the spacial occupation of the pioneer bees at Burst7. The left top heat map describes the spacial occupation of the pioneer bees at Burst28. The right middle heat map describes the spacial occupation of the pioneer bees at Burst43. The right bottom heat map describes the spacial occupation of the pioneer bees at Burst91.

In order to analyze the spatial distribution of pioneer bees in more detail, we ,first, separated pioneer bees into two groups. One group consists with bees which are foragers and pioneers(FP bee), and the other group consists with bees which are non-foragers but pioneer bees(NFP bee). Then we examined the spatial distribution of these groups in the hive. In pre-phase, the specific distribution in the hive cannot be confirmed, but FP bee and NFP

bee both stayed near the entrance in the first 1 or 2 days of the post-phase. From the second day onwards, the FP bee tends to remain near the entrance, whereas the NFP-bee tends to distribute inside the hive than FP-bee.

After the third day, the trend which bees gather at the entrance more remarkably appears, the FP-bee stayed near the entrance both before and after the burst, while the NFP-bee stayed in part near the entrance just before the burst, The tendency to be distributed inside the nest was frequently observed at decay of the bursts (Fig 4.12).

Also, we examined the differences in the amount of time spend to the trophallaxis between FP-bee and NFP-bee(Fig. 4.13). This result suggests NFP bees have a role in the information spreading to the inside of the hive.
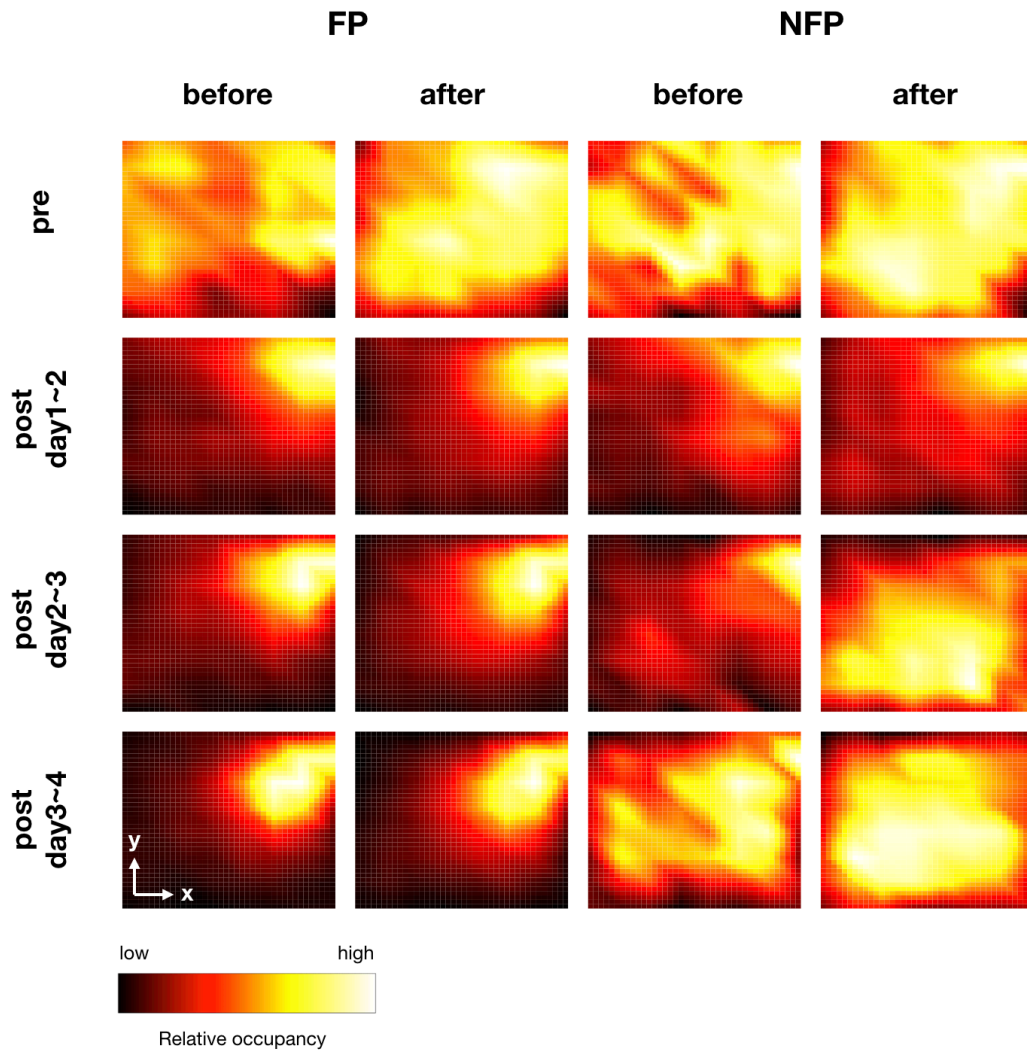
Figure 4.12: Spatial distribution patterns within the hive of FP bees and NFP bees as the average of the all trial. The location of the entrance located at the top-right side of each heat-map. "before" columns mean the spatial distribution pattern before the endogenous burst started. "after" columns mean the spatial distribution pattern after the endogenous burst's peaks. Color indicates relative occupancy, smoothed by kernel density estimation. FP bees tended to locate near to the hive entrance, but NFP bees tend to locate deeper area in the hive.
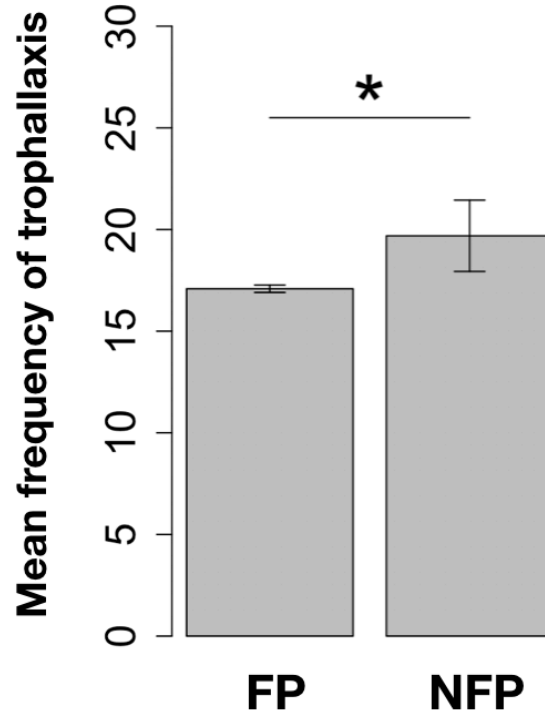
Figure 4.13: NFP bee spent a longer time to trophallaxis than FP bee. The p-value was determined by Brunner-Munzel test. $* < 0.1$.

4.3.3 Who are "pioneer bees"?

So far, we have examined the relations between some factors (kinetic energy, trophallaxis, waggle dance, and spatial distribution) of pioneer bees. In the end, we examined that which factors are mainly resposibble for the pioneer bees' behaviors. We calculated the five individual behavioral metrics: the average of K_i, the average time of foraging duration, the average time of waggle dance, and the average time related to trophallaxis for each. We applied the principal components analysis to reduce the dimensionality of this dataset (Fig. 4.14).

The first principal component (PC1) explained 37.2% of the observed variation and it was correlated with the foraging duration and the dance duration. These two factors were correlated with the aspects of foraging behavior. The second principal component (PC2) explained 27.5% of the observed variation and it was correlated with the trophallaxis and the kinetic

energy.

These two factors seem to correlate with the information spreading dynamics(Fig. 4.14.A). Both PC1 and PC2 were correlated with the distance from the entrance. Also, we found that lower PC1 scores and higher PC2 scores were both significantly correlated with the characteristics of pioneer bees(Fig. 4.14.C). Lower PC1 means higher foraging behavior and lower distance from the entrance. These results suggest that "pioneer bee" is mostly related to the foraging behavior but also the information spreading.
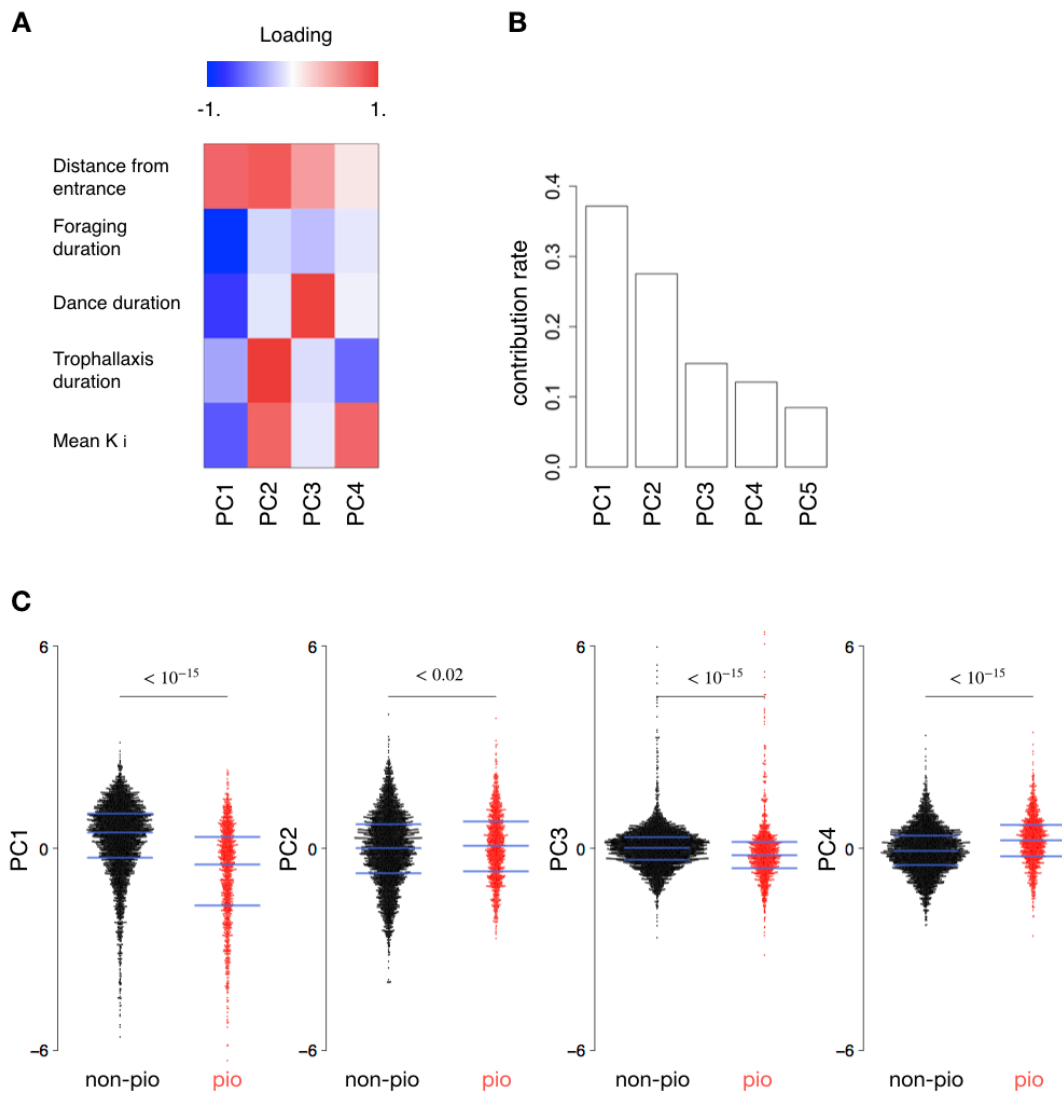
Figure 4.14: (A) Loading of individual behavioral values on Principal Components 1,2,3 and 4. The colors describe direction and strength of loading. (B) The cumulative contribution ratio of Principal Components 1,2,3 and 4. (C) Beeswarm plots of each Principal Components by pioneer bee status (non-pioneer vs. pioneer). The points describe data of individual bees, and thick lines show 75%, median and 25% values (from top to bottom) for each group. The p-values were determined by Brunner-Munzel test.

4.4 Discussion

We speculated that the bees which become active before a peak of an endogenous burst trigger the endogenous burst from the results of the previous chapter. We named these bees "pioneer bee" and we used non-negative factorization to identify pioneer bees.

Our interest is whether these pioneer bees are determined randomly or based on a kind of rules. To clarify this, we classified each burst based on pioneer bee who participated in each burst using a dimension compression method(nMDS).

Figure 4.5 showed the bursts which occurred before the entrance of the hive was opened (pre-phase) and the bursts which occurred after the entrance of the hive was opened (post-phase) constitute clusters in the MDS space. This result suggests that the pioneer bees group at pre-phase and the pioneer bees group at post-phase are different. Also, we analyzed the relationship between forager and pioneer bees and found that, after the hive entrance was opened, more than half of the pioneer bees were forager bees.

On the other hand, the exogenous bursts tend to aggregate or be plotted the different place from the the cluster of the endogenous bursts. The number of the pioneer bees of the exogenous bursts are smaller than that of the endogenous bursts so that the elements of the pioneer bee matrix of the exogenous bursts are almost 0. Therefore the Jaccard distances between each exogenous burst are small. Thus, the exogenous bursts tend to aggregate on the same place of the nMDS space. Also, about the pre-phase, some exogenous bursts are plotted in the cluster of the endogenous bursts. We speculate that there is no particular decision mechanism of the bee becoming a pioneer but bees that are sensitive to environmental fluctuations become pioneer bees. Therefore, these "sensitive" bees also tend to become the pioneer at the exogenous bursts.

Gernat *et al.*(2018) reported that within their identified trophallaxis network, "information" spread faster than it could in a randomly connected network. It is known that foraging bees are involved in the trophallaxis network, and this study showed that pioneer bees could cause bursts. Since our study showed that around half of the pioneer bees are foraging bees, we can speculate that the trophallaxis network may be related to the burst dynamics. Previous work has suggested the possibility that trophallaxis plays an essential role in intracellular signal transduction. A forager bringing food from outside the nest is involved in trophallaxis, and even the non-forager bee (for example, a bee that receives the nectar from the forager and stores the nectar to the hive called "nectar receiver") contributes significantly to the trophallaxis network.

Figure 4.9 shows that bees which spend their time on trophallaxis above the average are more frequently involved in as pioneer bee. Also, Figure **??** shows that there is a tendency to become a constituent factor of pioneer bee, even for non-forager whose activity related to trophallaxis is larger than the average.

What kind of spatial distribution does pioneer bee show in the hive?

We separated the pioneer bees into "forager pioneer bee" (FP-bee) and "non-forager pioneer bee" (NFP-bee) and investigated the difference of the spatial distribution of each burst(Fig. 4.12). At pre-phase, we also could not detect the positive difference of the spatial distribution of both pioneer bees. At post-phase, both FP-bees and NFP-bees existed near to the hive entrance, but at the end of the post-phase, the distribution of NFP-bees sifted to middle of the hive though FP-bees still existed near to the entrance. Furthermore, the spacial distribution of NFP-bees after a burst were the more inside of the hive, in contrast, the the spacial distribution of FP-bees after a burst were still near to the entrance.
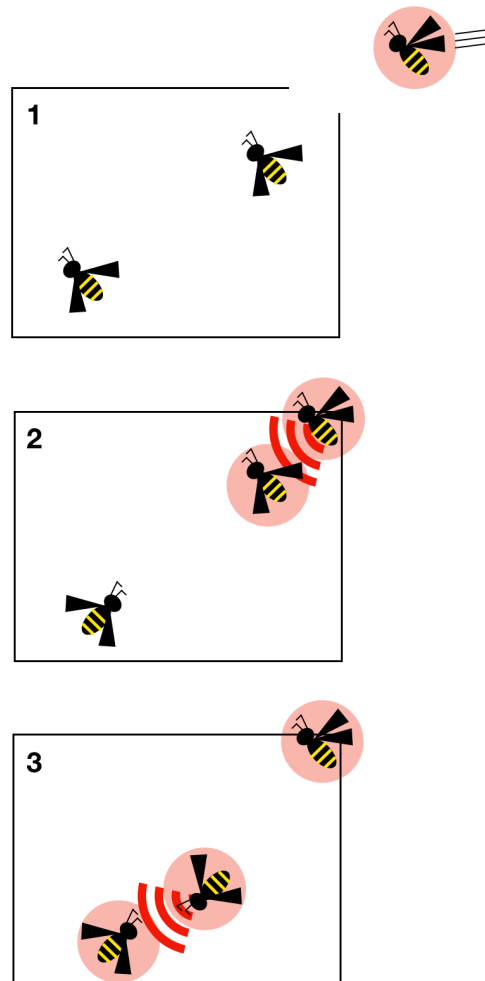
Figure 4.15: Illustration of the the possible mechanism of bursts. Red circles describe active bees and red wave-like marks describe the physical/vibration contact.

Because individuals may not experience external environment directly except for foragers, but instead rely on information received from foragers, the honeybees may be particularly responsive to social entrainment. Our findings suggest that pioneer bees are categorized into two types. One is the bees related to the so-called foraging behavior that brings some information from the outside into the nest, and one is a non-forager bee that is enhanced by returned forager and spreads the information to inside the hive (Fig.4.15). Figure 4.14 reinforces this suggestion because lower PC1 explained the bees which were involved in the foraging behavior and their distribution is near to

the entrance, and also higher PC2 explained the bees involved in trophallaxis behavior and showed higher K_i which seemed to relate to the medium for the bursts and their distribution is the inside of the hive.

In this study, we only investigate the bursting behavior detected by Kleinberg ' s burst detection algorithm. However, there were small burst like activities which was not detected by Kleinberg ' s algorithm. We speculate that there are some reasons that these small bursts does not grow to larger global bursts. By examining these small bursting behaviors, we may reach more detailed mechanism of the bursting behavior.

Chapter 5

General discussion

5.1 Function of burst for organized collective time and behavior

Donald O. Hebb was the first thinker who declared that the ability to produce consistent thinking of the brain comes from neurons' spatiotemporal orchestration[73]. This hypothesis is the "cell assembly" hypothesis.

A cell assembly is a group of functional cells that are mainly involved in the expression of a single perceptual/memory object in the cerebral cortex. The network of the brain before development does not have a functional structure. It functions only according to the statistical and physical characteristics of network coupling. Therefore, the activity of the cell group does not have functional meaning.

Hebb assumed the presence of a mechanism that strengthens the synaptic connections between cells that fire with a temporal correlation with each other. He believed that the selection and immobilization of the cell population, which simultaneously enhance the firing activity, are self-organized by repeated stimulation from the outside (Hebb's law). Once such a cell assembly is structured, its function will be maintained even when its neurons are replaced.

Diesmann and colleagues revealed that information can be stably propagated while maintaining time accuracy by synchronizing individual nerve activities[35]. This phenomenon is termed the synfire-chain and is assumed to play a role in stabilizing the cortical circuits.

In the case of animals acting in groups, for example, starlings and sardines are well-known examples of groups that act as super-individuals[3]. By forming groups, they reduce the chance of being attacked by predators. If they are not organized and gather in small numbers only, then they cannot

easily avoid predators[74][75].

Furthermore, time synchronization within groups is essential for acting as a group. The sharing of group time in a hive is important for the survival of honeybees, who live in an environment where the majority of the population cannot see the sun. Such group functions are likely to be posterior-organized due to interaction with the environment rather than determined congenitally. How does an animal group train/organize functions as a group?

The burst behavior of honeybees that we discovered may be thought of as a "rehearsal" for the bees to generate time as a flock and to act as a flock.

5.2 What is the relation between the burst and the circadian rhythm?

Our findings suggested that bursts often occur during the day, as shown in the figure 2.6 and by the fact that the pioneer bee is related to foraging behavior. However, we did not identify any direct relationship between the circadian rhythm and bursting behavior.

Figure 4.7, which shows the relationship between foragers and pioneer bees, and the analysis results of Trial 1202 revealed that the proportion of forager and pioneer bees (FP bees) remained low in comparison with those in the other trials. Moreover, according to Figure 2.6, the vertices of the diurnal cycle tend not to be within the orange frame showing daytime. Therefore, the phase tends to reverse during day and night.

We speculate that foragers' failure to work as pioneer bees may influence the synchronization of circadian rhythms throughout the hive.

5.3 Why did bursts occur before the entrance was opened?

The pioneer bees revealed that it is significantly related to the bees associated with the foraging behavior, especially after the hive entrance was opened. Meanwhile, before the entrance of the hive was opened, bees that identified as foragers had a tendency to become pioneer bees, but no significant difference was observed, and no spatial distribution was characterized.

At least after the entrance was opened, we can suggest that the burst had the function of spreading the information of the external environment to the nest-mates. That is, forager bees performing outside carried a kind of information to their hive, and these individuals entrained hive workers through

physical, short-distance interactions or fluctuations in the microenvironment. Entrained hive bees may socially synchronize with hive bees that have not encountered foragers.

What was the function of the burst before the entrance was opened?

Interestingly, in Trial 1202, not only the cycle of the circadian rhythm shifted from the diurnal cycle. The number of endogenous bursts before the entrance was opened was less than in the other trials.

One hypothesis inferred from this finding is that bursts in the pre-phase may have "warm-up-like functions" that cause organized global entrainment. In other words, Trial 1202 did not synchronize with the diurnal cycle synchronously throughout the hive even after the entrance was opened because it did not have sufficient "warming up" (endogenous bursts).

In the second half of this paper, we analyzed the nature of pioneer bees and demonstrated that at least foragers and pioneers had a significant relationship. However, how pioneer bees were determined at the pre-phase could not be clarified. We hypothesize that pioneer bees may be more sensitive to external stimulation than non-pioneer bees, and the summed activities of these cowardly pioneer bees gradually entrain all the bees in the colony.

5.4 Possible integration scenario

To summarize these suggestions and speculations, we could make one possible scenario.

1. Pre-phase: Training phase for synchronizing the entire hive. There is no particular decision mechanism by which bees become pioneers. Bees that are sensitive to environmental fluctuations become pioneer bees, and their activities spread to the hive in a chain. 2. Post-phase: The stage where the training phase is completed and the entire hive becomes easy to synchronize. The activities of foragers can be easily propagated throughout the hive, thereby allowing the entire hive to efficiently function as a single system.

5.5 Future works

The relationship between the frequency of bursts in pre-phase and the behavior of subsequent behavior of the hive (for example, more quickly formed circadian rhythms, etc.) must be closely followed to validate this scenario. In this study, all trials lasted approximately one week; longer-term experiments would be necessary to clarify the above relationship.

The experiment periods of the proposed data from our collaborator were summer. In general, bees perform foraging behaviors except during winter. We have to confirm whether our findings are observed in the other seasons. Bees are known to exhibit shivering behaviors to go outside their hives when outside temperatures are low. Such shivering behaviors may be related to the bursting behaviors.

Furthermore, the ability to suppress the burst of a hive in a certain manner, compared with burst-suppressed hives with normal nests, would enable us to explore the function of bursts in more detail. In addition, the addition of a disturbance (such as the replacement of individuals in a hive that has already formed sufficient social entrainment/collective time) and observations of the burst structure and changes in the constituent factors of pioneer bees may produce new insights into the robustness of collective time.

Studies on swarms of starlings reveal that even with changes in flock size, the rules behind the swarms are unchanged (so-called scalability)[76][77]. In this study, the individual densities within each hive were almost the same. Thus, we need to pursue scalability with a larger honeybee hive. In addition, the colony used by our collaborator was smaller than the typical wild colony (approximately 5,000 in size). Their tracking system can distinguish a maximum of 2,048 bees; hence, wild colonies cannot be individually tracked for now. Nonetheless, we need to confirm that our findings are observed in wild honeybee hives in future work.

Our findings also suggested that we can control the bursting behaviors of honeybees using micro robots, such as "Alice," as artificial pioneer bees[78]. By using artificial pioneer bees, we may identify the function of the burst in detail. In addition, Young *et al.* found that starlings' flocks consistently coordinate their movements with their seven nearest neighbors[79]. We speculate that there are efficient numbers of pioneer bees needed to entrain hives and facilitate the global bursting behavior. We may examine this concept of the efficient number to use artificial pioneer bees.

Chapter 6

Conclusion

Our study is the first report about the bursting behavior of the micro time scale in honeybee hives. Using massive data set of the approximately 5,000 individuals tracking data of honeybees, we found that honeybees showed bursting behavior which is one of the social entrainment and the time-scale is smaller than the circadian rhythm regarding their locomotion activities.

These bursts are quantitatively classified as endogenous or exogenous based on their causes. The features of exogenous bursts are sudden increases in $K_G(t)$, which happens because of external stimuli. On the other hand, the characteristics of endogenous bursts are gradual increases in $K_G(t)$, which happens spontaneously, potentially resulting from intrinsic bee interactions. Also, we demonstrate that the physical contact is one of the cause for the endogenous bursts by both the experimental analysis and the agent-based model simulation.

These results suggest that the bees which become active before the endogenous burst will become maximized trigger the endogenous burst. We named these bees "pioneer bee," and we used the non-negative factorization to identify pioneer bees.

Our interest is whether these pioneer bees are determined randomly or based on a kind of rules. We demonstrated the pioneer bees are not identified randomly but categorized into two types. One is the bees related to the so-called foraging behavior that brings some information from the outside into the hive, and the other one is a non-forager bee which is enhanced by returned forager and spreads the information to inside the hive. Figure 4.14reinforces this suggestion because lower PC1 explained the bees which are involved in the foraging behavior and their distribution is near to the entrance, and also higher PC2 explained the bees involved in trophallaxis behavior and showed higher K_i which seemed to relate to the medium for the bursts and their distribution is the inside of the hive.

As we described in Introduction, Bloch *et al.* has made four hypotheses about the possible mechanism for entrainment of circadian rhythms in honeybee colonies.

To summarize our findings, we suggest that, at least about the social entrainment of the bursting behavior, Hypothesis 2 or 3 is a possible mechanism for entrainment of the bursting behavior. Namely, the foragers(FP bee) carry a kind of information to their hive, and the foragers entrain hive workers(trigger the burst) through physical or close distance interactions. Then the entrained hive bees(NFP bee) synchronize with other hive bees that have not encountered foragers.

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Acknowledgement