

# Clustering experiments

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**Abstract** It is well known that bees cluster together in cold weather, in the process of swarming (when the “old” queen leaves with *part* of the colony) or absconding (when the queen leaves with *all* the colony) and in defense against intruders such as wasps or hornets.

In this paper we describe a fairly different clustering process which occurs at any temperature and independently of any special stimulus or circumstance. As a matter of fact, this process is about four times faster at 28 degree Celsius than at 15 degrees. Because of its simplicity and low level of “noise” we think that this phenomenon can provide a means for exploring the strength of inter-individual attraction between bees or other living organisms.

For instance, and at first sight fairly surprisingly, our observations showed that this attraction does also exist between bees belonging to *different* colonies.

As this study is aimed at providing a comparative perspective, we also describe a similar clustering experiment for red fire ants.

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*Preliminary version, comments are welcome*

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## Introduction

The paper describes a clustering process through which spatially dispersed bees aggregate into a single cluster. This is similar to the condensation process through which molecules of vapor come closer together to form droplets of liquid water. We think that such clustering processes may give information about inter-individual coupling strength in a population, just like data about phase transition provide information on inter-molecular interactions.

After the present introduction there will be three parts in this paper. (i) First we describe a typical clustering experiment so that such experiments may be repeated (and possibly expanded) by other researchers. (ii) Secondly, we give some preliminary results. (iii) Finally, we list a number of questions which we plan to explore in forthcoming experiments.

But before we start let us answer a fairly natural question, namely what is the rationale for *measuring* coupling strengths in animal or human populations, which indeed is our final objective. It is fairly obvious that the bond between children and their parents is stronger than the link between colleagues working in the same company but is it two times or ten times stronger? We do not know because, so far, there is no experimental procedure for probing the strength of such links<sup>1</sup>. This simple example emphasizes the fact that in contrast to physicists, biologists or sociologists have no quantitative knowledge whatsoever about interaction strength.

But why is it important to explore the strength of bonds? From physical chemistry we know that intermolecular coupling strength is the determining factor of most physical properties of any compound. For instance, as is well known, water molecules will form vapor, drops of liquid or blocks of ice depending on the coupling strength between them. In these three states the mechanisms of interaction between molecules are basically the same, only their average distances and their spatial organizations differ. The same conclusion holds for many other important physical properties, e.g. density, boiling temperature, latent heat, equilibrium vapor pressure, speed of sound. In short, coupling strength is key to characterizing the main properties of a system.

Populations of insects (or of small animals such as fruit flies or small fishes) provide a convenient testing field for experimental methods and devices designed for measuring coupling strength. Intuitively, one would expect social insects to have stronger

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<sup>1</sup>Let us emphasize that the time scale does matter here. It would be fairly meaningless to try to answer such a question on a daily basis because there would be big (and more or less random) fluctuations. On the contrary, on a time scale of several decades, the connections inside a family are likely to be maintained with more stability than those between colleagues. In addition to time averaging there should also be an average over a sufficiently large population. For measurements of inter-molecular interactions these averaging processes are done, so to say, automatically because any sample will include of the order of  $10^{23}$  molecules and the time-scale of the fluctuations in their interactions is of the order of  $10^{-10}$  second.

bonds than insects which do not live in colonies. As it is probably easier to measure strong bonds rather than weak bonds, it may be a good strategy to begin by studying insects which have strong social organizations such as honey bees or some species of ants.

That is why our attention was particularly attracted by a paper published in 1950 by a French naturalist (Lecomte 1950) which describes a clustering process in a (sufficiently large) population of bees. The first step in our investigation was to repeat this experiment. This is described in the next section.

## Description of a clustering experiment

The experiment described below was performed on 16 November 2011 at the “Eastern Bee Institute” of Yunnan Agricultural University, Kunming, China.

(1) At around 1pm during a sunny and fairly windy afternoon some  $n = 120$  bees<sup>2</sup> were transferred by aspiration from the frames of a beehive into a plastic bottle.

(2) Immediately after being collected the bees were put to sleep by flooding the bottle with carbon dioxide during about 2 minutes.

(3) Then the bees were spread on the bottom of a box of size ( $a=22\text{cm}$ ,  $b=29\text{cm}$ ) as shown in Fig. 1. In this experiment we did not try to distribute the bees uniformly on the vertex of a lattice because we wished to follow the same procedure as described by Lecomte (1950).

This repartition corresponds to a density  $d = N/S = N/ab = 18.7$  bees per square decimeter. A more suggestive parameter is the average spacing,  $e$ , between closest neighbors. For a density  $d$  each bee occupies an area  $s = S/N = 1/d = 5.3$  square centimeter. In other words, the average distance between neighboring bees is  $e = \sqrt{s} = 1/\sqrt{d} = 2.3\text{cm}$ .

(4) The box was put outside in sunshine but closed with a board of wood so that the bees were in the dark. The experiment started at this point. Time was 1:40pm. Every 10 minutes the box was opened for a short moment (around 20 s) for a picture to be taken. The temperature inside of the box was monitored thanks to a digital thermocouple thermometer. On average it was around 28 degree Celsius with an upward trend due to the sunshine.

(5) At 1:50pm there were about 8 small clusters comprising 7 to 15 bees; some 25 “isolated” bees were not yet part of any cluster.

(6) At 2pm there were 4 clusters as shown in Fig. 2. Each of the two large clusters had some 35 bees whereas the two smaller clusters had 15 bees. Clearly, the reduction by a factor two in the number of clusters was achieved through the

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<sup>2</sup>*Apis cerana* also called Eastern honey bees. The *cerana* bees have an average body length of about 12mm which means that they are slightly shorter than western honey bees (*Apis mellifera*) which on average have a length of 14mm.



**Fig. 1: Clustering experiment: initial distribution of bees.** Some 120 *cerana* bees have been put to sleep with carbon dioxide and spread on the bottom surface of a container. The average spacing between nearest neighbors is 2.3cm. The temperature in the container is around 28 degree Celsius.

coalescence of neighboring clusters.

(7) At 2:10pm, that is to say, half an hour after the experiment started, only two clusters remained: a big one which was moving (globally) on one of the side walls of the box and a much smaller one which remained at the bottom.

(8) When the box was opened again at 2:20 a large number of the bees flew out of the box. In this respect it should be noted that even shortly after the start of the experiment a few bees managed to fly away<sup>3</sup>.

## Results

The present paper summarizes only the first phase of our investigation, but, however limited, the experiments that we have conducted so far lead us to three useful conclusions.

- (1) The aggregation effect can be observed repeatedly with only small variability.
- (2) This aggregation effect has nothing to do with the cluster formation that occurs

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<sup>3</sup>For a physicist this does not come as a surprise. Of course, if one wishes, one can try to imagine some special reasons. For instance, it may be that the repartition of carbon dioxide in the bottle was not uniform with the result that some bees did almost not sleep. But one can also take a more global perspective by observing that some molecules are able to escape even from a block of ice as shown by the fact that for ice at -10 degree Celsius the equilibrium pressure is 2.6 mbar. This means that  $2.6/1000 = 0.26\%$  of the molecules have been able to escape. For the sake of comparison at +10 degree the vapor pressure is 12 mbar, that is to say only 5 times higher than over ice.



**Fig. 2: Clustering experiment after 20 minutes.** In the process which is under way the bees had first aggregated into many small groups and then these groups have been moving toward one another to form larger clusters until forming one single cluster at the end of the experiment (as shown below in Fig. 3).

in cold weather.

(3) The present aggregation phenomena gives a key for probing the attraction strength between bees or other living organisms.

The first point is perhaps of particular importance because biological experiments are often plagued by a high level of noise.

### **Speed of clustering increases with temperature**

When we discussed the experiment described in the paper by Lecomte (1950) with other researchers several of them suggested that the clustering effect may be a reaction to low temperature. This was indeed a natural comment for it is well known that bees cluster to increase the temperature in the middle of the cluster. In such cases they do not only form a cluster but they also activate their muscles (and especially the strong muscles of their wings) so as to generate heat. Such a clustering may have different purposes.

- One purpose is to keep the temperature of the brood at the required level of 35 degree Celsius in spite of a temperature outside of the beehive which may be much lower.
- A different clustering purpose is a defense tactic against hornets or wasps. When a hornet or a wasp tries to break into a beehive of *cerana* or *mellifera* a ball of bees

surrounds the invader. The bees vibrate their flight muscles until the temperature inside of the ball is raised to 47 degree Celsius. Together with an elevated concentration of carbon dioxide this temperature kills the intruder but does not harm the bees because their lethal temperature is higher. It can be noted that fever in humans is a similar tactic against viruses<sup>4</sup>.

In these two cases, the purpose of the cluster is to provide thermal isolation and to concentrate the production of heat. In order to test whether or not the clustering described by Lecomte is of the same type we performed the same experiment at two different temperatures.

- A first experiment was conducted at 15 degree Celsius. Clustering occurred but took a long time, about 2 hours. At such a low temperature one might at first suspect that the clustering was indeed an attempt to keep heat inside of the cluster. However, the bees did not vibrate their flight muscles which means that they did not try to raise the temperature of the cluster.

- A second experiment was done at 28 degree Celsius. As this temperature is already higher than the normal temperature of the bees in the beehive (that is to say the temperature between the frames) there is certainly no need to cluster to prevent a loss of heat. Nonetheless, clustering occurred and was in fact about 4 times faster than at 15 degree.

### **Clustering in a mixed population**

In this experiment, some hundred *cerana* bees were collected from a beehive *A*, and another hundred from a beehive *B* located some 15 meters from *A*. All the *A* bees were marked with a white dot. Then, the two populations were put to sleep and dispersed at the bottom of a box as explained in the previous section. The box had an area which was twice the area of the previous box so as to keep the same population density. The question was whether the *A* and *B* bees would form separate clusters or whether they would form mixed clusters.

It is a common belief that each colony has its own odor and that if a foraging bee from *A* tries to enter the beehive *B* it is identified at the entrance, prevented from entering and possibly even killed. One of the US researchers with whom we were

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<sup>4</sup>The account of this defense tactic given in the Wikipedia article entitled *Apis cerana* (English version) is not really reliable in several respects. First, he does not cite the original paper by M. Ono, I. Okada and M. Sasaki (1987). Secondly, it says that this defense tactic is specific to the *cerana* bee while in fact, as shown by K. Tan et al. (2005), it is also used (albeit less effectively) by the *mellifera*. Thirdly, it does not mention the role of oxygen deprivation which was shown to be an important factor by M. Sugahara and F. Sakamoto (2009); moreover it gives the lethal temperature of *cerana* bees as being 49 degrees while it seems closer to 51 degrees according to the same authors. Finally, it does not mention that the same kind of defense is used not only against giant hornets but also against wasps (see K. Tan et al. 2005, 2010). Incidentally, what is not completely clear in the defense tactic against the giant Japanese hornet is why the hornet does not simply kill the bees which surround it in order to break this deadly surrounding. Indeed, the Wikipedia article about this giant hornet says that it can kill as many as 40 bees per minute. Thus, it should be able to “drill” an escape tunnel through the ball in less than one minute, that is to say much less than the 10 mn that it takes for it to be killed by the ball.

in contact before doing this experiment emphasized the crucial role of odors in the communication between bees.

Yet, observation instead showed that the bees form a mixed cluster (see Fig. 3). This



**Fig. 3: Mixed cluster of bees.** The bees from colony *A* are marked with a white dot whereas those of colony *B* have no dot. The picture shows that the cluster comprises bees from the two colonies.

result is not completely unexpected because beekeepers know that it is possible to put frames from different colonies into the same beehive. Fights may be prevented by spraying all the bees with flavored water.

### **Possible role of vibrations**

In his paper of 1950, J. Lecomte describes a second experiment which is the following. On the bottom of the box where the bees were scattered, he disposed a cell containing at least 100 bees. Then he found that, once formed, the whole cluster would slowly move toward this cell (at a speed of about 6 cm/hour) until covering it completely.

In a connected paper Lecomte (1949) made an observation which may give a clue as to the interaction mechanism. He observed that if a wad of cotton is interposed between the cell containing the bees and the bottom of the box the cluster does no longer converge toward the cell. Lecomte attributes this result to the fact that the vibration of a frequency around 30Hz that the bees are known to produce is damped by the cotton. In his article of 1950 he says that he tried to simulate this effect by generating a vibration of same frequency with an electro-mechanical device but it failed to attract the cluster of bees.

### **Role of the number of bees**

In a subsequent article published in 1956, J. Lecomte investigated the role of the number of bees in a more quantitative way than he had done in his papers of 1949

**Table 1 Influence of the number (and density) of bees on cluster formation**

Number of bees	5	10	15	25	50	75
Frequency of formation of a cluster	30%	40%	6%	6%	80%	100%

Notes: The experiments were performed at 25 degree Celsius. For each total number of bees the experiment was repeated 18 times. The same box was used in all experiments which means that the density of the bees per square centimeter decreased along with the number of bees. A cluster was defined as an aggregate containing at least 80% of the total number of bees.

The results show a fairly sharp transition between 25 and 50 bees.

There is no clear explanation for the fact that the probability increases again for 5 and 10 bees; of course, for such small numbers the variability may be large which means that in addition to the average one would also need to know the standard deviation.

Source: Lecomte (1956).

and 1950. He carried out 6 series of experiments with 5, 10, 15, 25, 50, 75 bees respectively. In each series the experiment was repeated 18 times which resulted in a total of  $6 \times 18 = 108$  experiments. He considered that a valid cluster had to include at least 80% of the bees. With this definition he obtained the results given in Table 1.

What is not really satisfactory in this experiment is the fact that the number and the density of bees change together. In order to determine which of these two variables is the determining factor, one needs to perform two series of experiments.

- In one series the spacing between the bees would be kept constant but their number would be decreased until the clustering process disappears.
- In a second series of experiment the number would be kept constant but the spacing between the bees would be progressively increased until the clustering process disappears.

This requires a fairly broad study that we are planning to conduct in the near future.

## Clustering experiment for ants

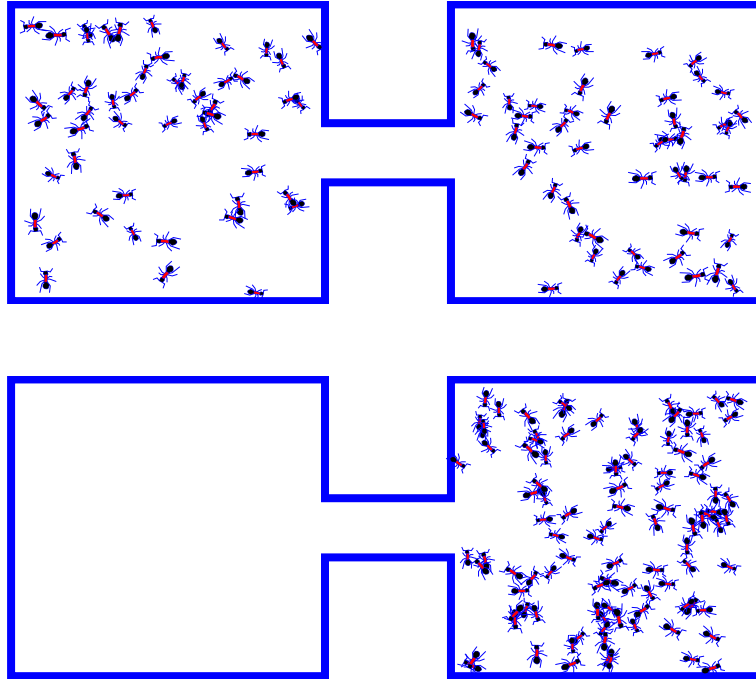
In order to emphasize our commitment to comparative analysis we describe in this section a clustering experiment involving ants. It was carried out with red imported fire ants (*Solenopsis invicta* Buren) in the summer of 2011 at the “Laboratory of Insect Ecology” of the South China Agricultural University.

### Description of the experiment

The experiment involved two steps.

- First a number  $N$  of ants were put in two flat boxes connected by a glass tube (Fig. 4). The boxes had an area of about 60 square centimeters. The connecting glass tube had a length of 3 centimeters and a diameter of one centimeter. The ants were initially put to sleep by using carbon dioxide. Thus, the ants were in a similar





**Fig. 4: Clustering experiment for ants.** The diagram at the top shows the initial state while the diagram below shows the state after a few hours when all ants have gathered on the same side. The ants came from the same colony. They were initially put to sleep through carbon dioxide. For the clarity of the diagram only 50 ants are shown on each side in the initial situation. In the experiment the initial numbers  $N$  of ants on each side were 100, 200, 300, 500, 750, 1000.

If  $T$  denotes the delay after which the ants are all in the same box one observes that the larger  $N$ , the longer  $T$ ; the relation between the two variables was found to be ( $N$  in hundreds,  $T$  in hours):

$$T = aN + b, \quad a = 0.5 \pm 0.3, \quad b = 2 \pm 1$$

with a coefficient of linear correlation of 0.84.

condition as the bees in the previous experiments.

- After a time of the order of several hours the ants gathered together on the same side. It can be observed that although the longest experiments lasted of the order of 12 hours, the ants received no food nor water during the experiment.

Of course, a few ants remained in the initial box; for instance the ants which did not awake after being put to sleep; the criterion which was used to terminate the experiment was that the number of remaining ants should be smaller than 10% of the number initially contained in the box.

## Results

For each  $N$  the experiment was repeated 5 times. The variability was fairly high; the coefficient of variation,  $\sigma/m$ , of the times in the 5 repetitions was on average 65%.

For each of the  $N$  the average of the 5 repetitions gave the following results ( $N$  in

hundreds and  $T$  in hours):

$$N, T : (1, 2.3) (2, 2.1) (3, 5.1) (5, 2.8) (7.5, 5.9) (10, 6.8)$$

The relation between  $N$  and  $T$  was found to be:

$$T = aN + b, \quad a = 0.5 \pm 0.3, \quad b = 2 \pm 1 \quad \text{for } 100 \leq N \leq 1,000$$

## Comments

Of course, for the sake of comparative analysis, it would be interesting to perform exactly the same experiment as with the bees, that is to say observing what happens when the ants wake up after being scattered on the bottom of a box. This is an experiment that we intend to do in the near future.

We are also planning a number of additional experiments.

- What happens when the number  $N$  becomes much smaller than 100, for instance of the order of 20 or 30. For bees, one knows that there is no longer any clustering for such small numbers. Is there also such a critical threshold for ants?
- What happens when ants from a colony  $A$  are introduced in one box and ants from a colony  $B$  in the other box? Will they nevertheless gather together in the same box?
- What happens when one uses a longer glass tube, for instance of a length of 30 centimeters instead of 3 cm? Will the ants nevertheless gather on the same side<sup>5</sup>?

## Forthcoming research

Before turning to the future, we give a short account of the research conducted in past decades, mostly in the time interval between 1940 and 1975.

### Past research

The papers published by J. Lecomte were not an isolated research but were part of a broader investigation into the collective behavior of social insects. At that time this research was conducted by a group of French scientists lead by Prof. Rémy Chauvin (1913-2009). Chauvin's interest into collective phenomena started with his PhD thesis (1941) which he devoted to the phase transition through which solitary locusts come to form large swarms containing up to several billion locusts<sup>6</sup>. In the four decades between 1940 and 1980, as the head of different laboratories, Chauvin and his students and collaborators investigated several facets of collective behavior

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<sup>5</sup>Preliminary observation shows that if the glass tube is longer, many ants will stay in it which will hinder passage of the other ants from one side to another.

<sup>6</sup>A comparative description of this phase transition for both animal and human populations can be found in Roehner (2005, 674-675).

among social insects.

In many respects the case of Rémy Chauvin was similar to that of the American psycho-sociologist Stanley Milgram. Like Chauvin, Milgram investigated collective behavior and he too saw his work decried in some circles of academia<sup>7</sup>. Unfortunately, both Chauvin and Milgram had few (if any) followers.

## The future

Future research can be directed into two different directions.

- One is the investigation of the *detailed* mechanisms that play a role in the clustering phenomenon for bees.
- An alternative direction is to make a *comparative* study of clustering phenomena in various conditions and for different species.

Of course, it is impossible to predict in advance which direction will prove the most fruitful. However, physicists would make the observation that in physics it was the second option which proved the most useful. It is easy to explain why if we consider what would be the parallel of the first option for physical systems.

Molecules have many mechanisms of interaction: covalent bonding, ion-ion bonds, dipole-dipole interactions, interaction through induced dipoles (the so-called London forces). A real investigation of these interactions requires that one study them not only at molecular level but also at the level of atoms and electrons. At this level the explanations must rely on quantum mechanics. In other words, in this direction one faces very difficult and tricky problems. One can even say that the answers will remain fairly elusive because there will always be a more detailed level to be considered.

In the same way, let us see what would be the second option for physical systems. It consists in lumping together all the different kinds of interactions and estimating the strength of the global attraction forces for instance in terms of the energy (expressed in electron-volts) that it takes to break such bonds. In this way, as already mentioned at the beginning of the paper, one is able to predict many important properties of a great number of liquids or solids.

It may be argued that an argument that holds for physical systems may not necessarily be true for biological systems. We will not know until we have tried. In this connection it can also be observed that the comparative approach that we advocate

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<sup>7</sup>The article that the English version of Wikipedia devotes to Rémy Chauvin does hardly justice to his scientific work in the sense that it focuses almost completely on Chauvin's researches about parapsychology and unidentified flying objects that he conducted in the last part of his life. Why should such topics not be open to legitimate scientific investigation? The attitude reflected in this article rather seems to be a sad reflection on the level of conformism and narrow-mindedness in some areas of present-day scientific research. Chauvin was also a vocal critic of neo-Darwinism because he saw it as an insufficient yet unfalsifiable theory. A more honest account of Chauvin's work can be found in the French version of the Wikipedia article.

here was commonly used (both in biology and in the social sciences) in the late nineteenth and early twentieth centuries. It is probably the high degree of scientific specialization that prevails nowadays that killed comparative research.

Just as one example of the kind of questions that one would like to consider in the future one can give the following illustration.

When one opens a beehive one sees hundreds of bees closely packed together on each frame. For *cerana* bees their spatial density may be of the order of 200 per square decimeter on each side of a frame. To our best knowledge this is a far higher density that can be found in a colony of ants<sup>8</sup>. Therefore one would expect a higher attraction force between bees than between ants. The challenge is to design an experimental procedure which gives meaningful attraction estimates in the two cases. It is known that for some species of ants there are also clustering phenomena<sup>9</sup>. Can they be used to get interaction estimates?

Although the analysis of clustering is only one among several possible methods for measuring interaction strength<sup>10</sup>, it seems to be one of the most promising. We hope that this paper will bring about more researches in this direction.

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<sup>8</sup>However it can be noted that in laboratory colonies of red fire ants there is also a high density of ants especially around the water supply.

<sup>9</sup>Many thanks to Dr. Lei Wang for the information that he gave us about a clustering experiment with red fire ants that he has conducted. In this experiment an equal number of ants is put in two boxes connected by a glass tube. After a few hours all the ants are concentrated on only one side.

<sup>10</sup>For indications about some other methods see Roehner (2008, chapters 4 and 5).

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<sup>11</sup>*Apis mellifica* is the same species as *Apis mellifera*. In Latin the meaning of "mellifera" is "to bear honey" whereas the meaning of "mellifica" is "to make honey" which is of course more correct because indeed bees absorb nectar and deliver the honey only once they come back to the beehive.