How can one measure group cohesion?

From individual organisms to their interaction

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Invited talk given at the COST[1] Workshop held in Galway (Ireland), 25 April 2014, 9:30-10:30. [2].

Abstract Measuring atomic and molecular interactions was one of the main objectives of physics during the past century. It was an essential step not only in itself but because most macroscopic properties can be derived once one knows interaction strengths. At the present time, except for systems that can be described as discrete networks (like the Internet network) our knowledge of social and biological ties still remains very limited. An important step is to develop experimental means for measuring social and biological interactions. In this talk there are two parts.

- Firstly, we describe experimental evidence of inter-individual attraction in populations of insects.
- Secondly, we focus on a specific system, namely populations of *Euglena gracilis*, a green, swimming unicellular organism, for which we try to determine individual and interaction properties.

Version of 13 June 2014. Comments are welcome.

Websites which may be useful in connection with this topic:

- http://www.lpthe.jussieu.fr/∼roehner/bola.html [Papers on the physics of living populations]
- http://www.lpthe.jussieu.fr/∼roehner/expclust.html [Pictures of clustering processes.]

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[1] "COST" means **Co**operation in **S**cience and **T**echnology. It is a program supported and funded by the European Union. Entitled "Ways of seeing", the workshop lasted from 23 to 25 April 2014

[2] This paper is also available on the "arXiv" (Princeton) and "BOLA" (Beijing) preprint archives.

Contents

Part 1: Introduction

Rationale for experiments on insects and microorganisms

Group cohesion in physics

What holds a system of particles together? What are the laws which rule the transition from a state of high cohesion to a state of lower cohesion and vice versa?

Such questions have been of fundamental importance in physics and chemistry. As an illustration, one can mention that when in 1900 at the age of 21 Albert Einstein submitted his first paper to the prestigious German journal “Annalen der Physik” his purpose was to derive the characteristics of inter-molecular attraction potentials from data about capillarity properties of various chemicals. At that time, such an objective was shared by many physicists.

Then, during the 20th century, little by little our understanding and knowledge of

microscopic interactions progressively became more accurate.

Incidentally, it can be observed that in a sense, the fact that until the second half of the 20th century molecular mechanisms were out of direct observational reach was a chance because it allowed physicists and chemists to focus on global properties before becoming involved in the complicated details of molecular mechanisms.

Group cohesion in human systems

The same questions constitute also central issues for social systems. Yet, they are not often formulated in such a way. Consider the following examples.

- It is commonly recognized that high performance teams or companies are characterized by effective interactions, but how can such interactions be measured? One might be tempted to use surveys; however such surveys will be unreliable when team members do not feel free to tell the truth, a situation which is likely to occur when there are no trustful relations.
- Everybody understands that the relationship between parents and their children is (in some way that needs to be defined) "stronger" than the connection they may have with neighbors or colleagues. The later may in turn be stronger than the links they have with fellow citizens. Yet, we are unable to quantify the respective strengths of such interactions. Why is this so? Here is a possible answer.

Before one can talk about quantification is it not necessary to define more precisely what one wants to measure? In science definition and measurement usually go hand in hand. If we could set up an experimental procedure for measuring the strength of family bonds the very conditions of the experiment would spell out what the experiment is measuring. But can we set up an experiment involving parents, children or citizens? Clearly not for obvious ethical reasons.

However, it is possible to perform experiments on living organisms such as insects and microorganisms, all the more so because such experiments do no require to kill or harm these organisms. The present research is in line with previous pioneering studies based on insect models such as Costa (1997), Viswanathan et al. (2011), Suematsu et al. (2011), Nishimori (2012).

Characteristics of interactions: strength, range, duration

Before trying to characterize interactions let us show a picture which illustrates one of the main difficulties in this kind of investigation. The picture in Fig. 1 seems to suggest an imitation behavior. However, it is known that this behavior occurs in response to a predator. Therefore, how can one be sure that the caterpilars do not just react individually to the same danger signal?
An interaction is characterized by its strength and range. In biological systems it is

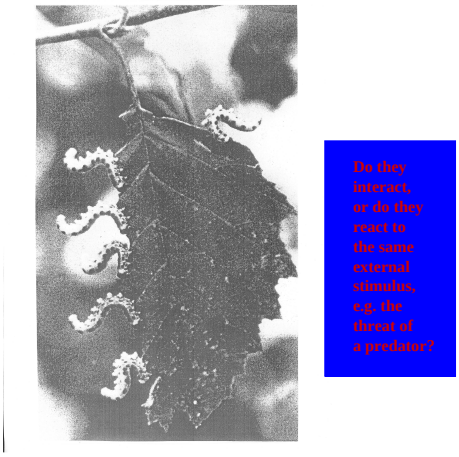

Fig. 1: Synchronous movements of caterpillars. The caption says that the picture "indicates that there is an exchange of information between individuals". This is not necessarily true, however. It can also be a common response to an external stimulus. The parallel orientations of iron filings sprinkled on a white card placed on top of a permanent magnet are not due to any interaction between them but solely to the external magnetic field. This illustrates what is probably the main difficulty when probing interactions. *Source: Costa (1997)*

is also characterized by its duration. Indeed, in contrast to physical systems in which interactions are permanent, systems of living organisms may have temporary interactions. The most obvious example is the interaction between males and females. It occurs only in mature animals and usually follows some seasonal pattern.

Classes of collective motions

By the term "collective motions" we wish to refer to the motions of many individuals in a population, whether they interact in some way or not.

Cases ranging from complete randomness to fairly ordered motions can be illustrated by the following examples.

(1) Independent motions of random individuals.

Ex: Motions of many Brownian particles in a liquid.

(2) Independent motions of deterministic individuals.

Ex: Movements of people on a square or the departure hall of a railway station. All persons know where they are going but the global picture is not very different from the former.

(3) Collective motion of non-interacting individuals: hydrodynamic interaction

When microorganisms swim fairly fast in water and when in addition their density is high enough the drift of the water induced by the movement of one individual will affect the movements of its neighbors. In this way, the trajectories may become correlated even though the organisms do not have any direct means of interaction. This effect is known as hydrodynamic interaction.

Ex: A spectacular example was studied by Rabani et al. (2013). In this case the bacteria were moving in a two-dimensional film of water which greatly enhanced

the hydrodynamic effect. As a result, individual velocities were fairly parallel to one another.

(4) Collision avoidance and the sidewalk effect

When ants come close to one another they usually stop for a few seconds in order to examine one another. Swimming microorganisms such as Euglena gracilis must of

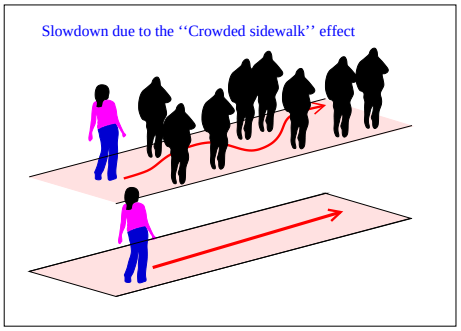

Fig. 2: Illustration of the “crowded sidewalk” effect. In a sense collision avoidance can be considered as being an interaction. It is similar to the kind of weak interaction that occurs between the molecules of a gas except that in a gas if one assumes the encounters to be elastic there is no slowing down effect.

course avoid collisions but their interaction when they are close to one another seems to be very brief. In other words, whether or not there is any “real” interaction there will be a slowing down effect due to the necessity of avoiding head-on collisions. We will see later on that this effect can be observed statistically.

(5) Attraction between individuals: clustering and shoaling

Ex: A shoal of fishes consists in a population of fishes remaining in the same location. Similarly, as we will see below, ants or bees left to themselves move toward one another and eventually form one big cluster. In the absence of any external stimulus this can hardly be explained otherwise than by an attractive force between them.

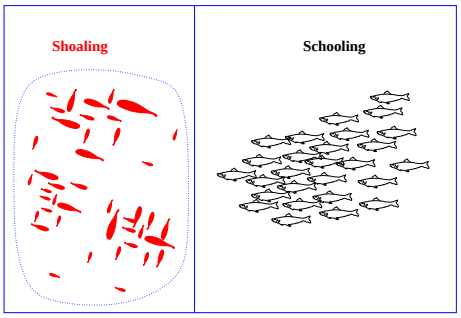

Fig. 3: Difference between shoaling and schooling. Shoaling means that a group of individuals remains in the same location. It is similar to clustering except for the fact that it can be a permanent situation whereas clustering is rather temporary. Schooling is similar but means that the group moves together. Both shoaling and schooling reveal an underlying inter-individual attraction. However, shoaling may also just reflect gathering in an appropriate environment.

(6) Collective motion of interdependent individuals

Ex: The V-shaped flight formation of geese or ducks or the collective motion of a school of herrings.

Part 2: Examples of inter-attractive behavior

In this part we focus on cases for which there is clear evidence of collective behavior brought about by inter-individual attraction forces.

Clustering as a means for estimating inter-attraction

The occurrence of clustering in a population *in the absence of any external stimulus* (whether light, food, smell or any other) provides good evidence for the existence of an attraction force, or more precisely it shows that the attraction force is strong enough to overcome the dispersion effect of individual and more or less random velocities. in what follows this dispersion effect will be referred to simply as noise.

The physical analog of clustering is the phenomenon of condensation (i.e. transition from a gas to a liquid) which is a (first order) phase transition. In this case, the noise is due to what is called thermal agitation. As the van der Waals interaction forces between molecules are basically the same whether the material is in gas or liquid state, condensation occurs when a reduction in temperature makes the noise kT become smaller than the attraction effect. This is a self-reinforcing effect because by reducing inter-molecular distances condensation at the same time increases interaction strength.

Observation of clustering provides in a fairly easy way an insight into the attractive forces between living organisms. That is why in the next section we will describe several cases of clustering. One should keep in mind that whereas clustering is evidence of attraction, the opposite is not true. Attraction without clustering simply means that the dispersion forces are too strong.

Attraction strength among social insects: ants and bees

First, we will study the clustering of ants and bees, i.e. two social insects. Then, we show that some non-social insects also form clusters. Naturalists call them gregarious insects. Finally, we describe the case of drosophila for which no clustering is observed. They are sometimes called solitary insects, but it is clear that even such insects have interactions, among which the attraction between males and females is the most obvious.

Ants

Fig. 4a shows that the behavior of ants is the exact opposite of a gas. Whereas a gas tends to occupy all the volume available, ants will cluster into one half of the container. As a matter of fact, they will not even occupy the totality of the right-hand half of the container but only a small part of it.

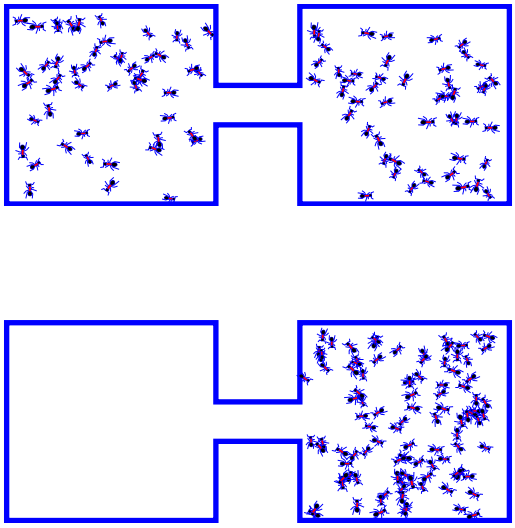

Fig. 4a: Clustering of red fire ants. The ants move from an initial state where they are on separate sides to a state where almost all of them are on the same side. The boxes had an area of about 60 square centimeters. The connecting glass tube had a length of 3 centimeters and a diameter of one centimeter. The experiment was done in August 2011 at room temperature that is to say at about 25 degree Celsius. The initial numbers N of ants on each side were 100, 200, 300, 500, 750, 1000. For each N the experiment was repeated 5 times. For these repetitions the coefficient of variation was on average 65%. Contrary to what happens for a gas the flow velocity does *not* increase when the density becomes higher. On the contrary it decreases. This means that the process is more and more dominated by attraction.
Just to give an order of magnitude of average traffic in the communication tube, it can be observed that for an initial population of 1,000 on each side the clustering process lasted 6.8 hours which means that the average flow of ants per minute was $1000/(6.8 \times 60) = 2.4$. This shows that the section of the communication tube was not in itself a limiting factor. *Source: The data are from an experiment done in August 2011 by Dr. Lei Wang at the South China Agricultural University in Guangzhou (personal communication).*

In the experiment of Fig. 4b the "edge difficulty", namely the fact that the ants tend to go to the edge of the container and stay there without moving at all was solved through the ring structure. In the experiment of Fig. 4c it was solved in a different way. The rectangular space in which the ants were spread was surrounded by 4 strips of paper which had been recovered with a repellent liquid whose smell the ants do not like. Before being used the paper strips were left to dry but the smell was still perceptible which means that they acted as a repellent[3].

Two qualitative rules emerge from these observations.

- The higher the density, the faster the aggregation process.
- When the density was low the cluster which appeared at long last comprised only a small fraction of the ants.

The graph of Fig. 4d documents the second effect. Observation shows that the

[3]However, when the space allocated to the ants was small, a fraction of them happened to cross the border. This occurred particularly at the start of the experiment when the ants were excited after having been removed from the rest of the colony.

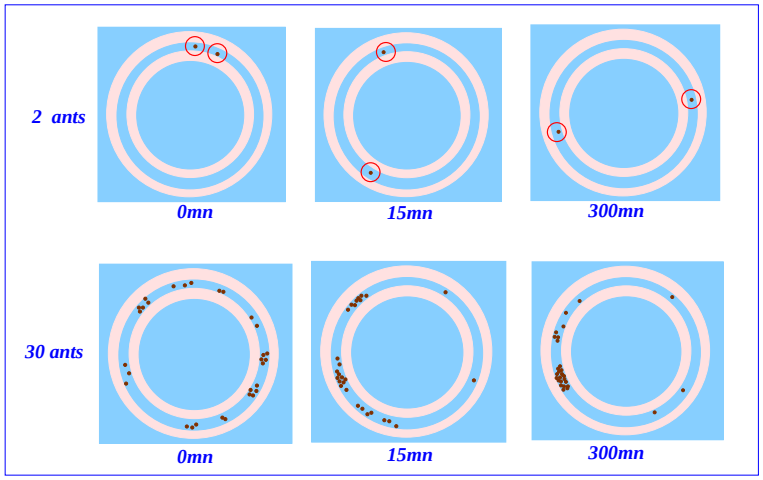

Fig. 4b: Clustering process for ants. The ants belong to the species *Terramorium, Caespitum*, Linnaeus 1758; their length is about 1-2mm; they were bought on Taobao, a well-known Chinese shopping website. The ring structure (made of polymer clay and covered by a plastic plate) prevents the ants from remaining motionless at the edge of the container as would happen with an ordinary container. The ability to form a cluster (as well as the speed of the aggregation process) depends upon the number of ants. The same observation holds for bees (see Table 1). Two ants on average remain fairly apart as shown in the top panel. On the contrary, for a group of 30 ants after a time interval of the order of one hour they form a cluster (or sometimes two clusters). In this experiment the diameter of the ring was 8cm. *Source: The experiment was done in March 2014 by Li Geng at the School of Systems Science of Beijing Normal University.*

dispersion (i.e. the proportion of ants not in the cluster) is higher in two dimensions than in one dimension, i.e. in the ring experiment.

Bees

Beekeepers know very well that bees cluster together under some special circumstances.

- In wintertime, when the temperature is low, they form a cluster[4]
- In the process of swarming, that is to say when the "old" queen leaves with *part* of the colony or in the similar process of absconding, that is to say when the queen leaves with *all* the colony.

The fact that bees form clusters *without any external stimuli* was discovered by J. Lecomte in the late 1940s. He explored carefully the conditions under which clustering takes place and published his results in three papers (1949,1950,1956) which, although an important contribution to the understanding of the *collective* behavior of bees, were completely overlooked and forgotten in the following decades.
Fig. 5b shows that clustering also occurs in a mixed population of bees coming from two different colonies.

[4]It is often said that it is for raising their temperature at least for those inside the cluster. However, it is also known by beekeepers that bee colonies start to become active in mid-February (in countries whose climate is similar to that of France) that is to say at a moment when the temperature outside of the beehive is still fairly low. It is in May and June that the production of honey is usually highest, not in July or August.

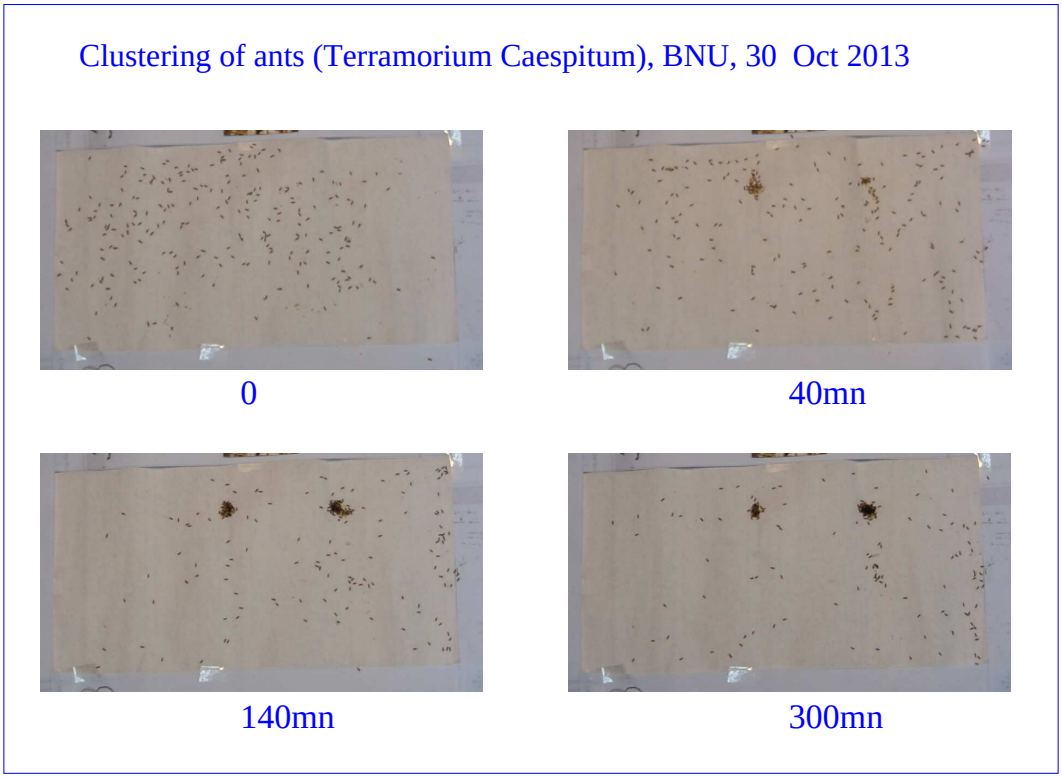

Fig. 4c: Clustering process for ants. Strips of paper impregnated with a repellent kept the ants away from the borders of the area. In this experiment there were 229 ants (density=57/sq.decimeter). Similar experiments were done with different densities and number of ants. An approximate rule of thumb that emerged is that the larger the number and density the less time it takes for big clusters to appear and the greater the proportion of ants which are in the cluster (see Fig. 4d). *Source: The experiment was done on 30 October 2013 and March 2014 at the School of Systems Science of Beijing Normal University.*

This 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. Nevertheless, it is also true that when a bee tries to enter into the beehive of a different colony is will be identified and chased away by the bees which stand guard next to the entrance[5].
The observations summarized in Table 1 suggest that for the formation of a single cluster there is a critical minimal population threshold which is of the order of 40 bees.

Attraction strength among non-social insects

Comparative experiment

First we describe an experiment that was done in parallel with drosophila and with beetles (Alphitobius diaperinus).

[5]Does this process occur systematically, that is to say with a probability of one, or rather with a probability $p < 1$, which would imply that some foreign bees can get entrance? This point is not clear from what we have read so far.

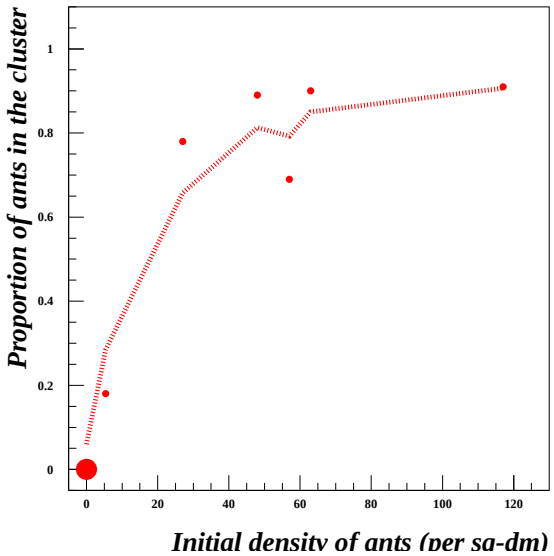

Fig. 4d: Relationship between density and the proportion of ants in the cluster. The small red dots are for experiments similar to those shown in Fig. 4c. A big dot was drawn at the origin because one can be certain that when the density tends toward zero the ants will be unable to find one another which means that no aggregation can occur. For the sake of simplicity *Source: The experiments were done in Oct-Nov 2013 at the School of Systems Science of Beijing Normal University* .

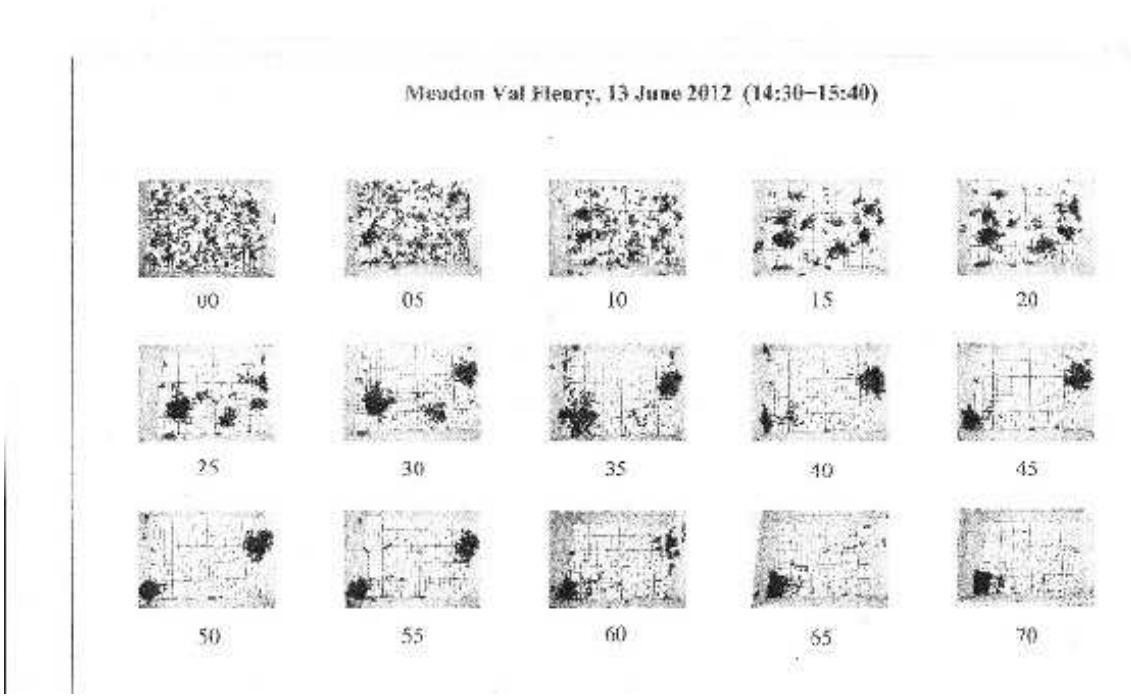

Fig. 5a: Clustering process for bees (Apis mellifera mellifera). Altogether there were about 300 bees. Initially they were put to sleep through 5 minutes in carbon dioxide. All the bees are from the same beehive. The numbers under the pictures give the time in minutes. *Source: The data are from an experiment done in Meudon Val Fleury near Paris in July 2012 by Jack Darley and Bertrand Roehner.*

One takes a test tube containing some 50 drosophila and one makes them all move

Fig. 5b: Mixed cluster of bees. It is often said that bees from different colonies do not mix with one another or may even fight one another. However, this experiment shows a case where they form mixed clusters. 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. *Source: The experiment was performed in December 2011 by Zengru Di, Bertrand Roehner, Ken Tan and Zhengwei Wang at the Eastern Bee Institute, Yunnan Agricultural University, Kunming, China.*

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 single 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).

to the bottom of the tube by hitting the tube on a table. Then, very quickly[6] one puts the tube on the table in horizontal position. Let us assume that the bottom of the tube is on the right. After a few seconds, some 5 flies will have reached the left-hand side, and may be 10 others will be in the middle of the tube. If one waits 5mn, the flies will be distributed fairly uniformly throughout the tube.

If one repeats the same experiment with beetles it will be seen that after 5mn almost all insects are still together on the right-hand side of the tube.

A physical interpretation of this experiment can be proposed.
Suppose that we replace the drosophila by the molecules of a gas and the beetles by the molecules of a liquid. By hitting the tube on the table all molecules will move

[6]This movement must be fast because drosophila have a natural tendency to go upward.

to the bottom[7]. For this experiment we do not need to put the tube in horizontal position; this was only required because of the tendency of drosophila to go upward. After the shock, the gas molecules will re-occupy the whole available room of the test tube within a fraction of a second.
In the case of a liquid, it will go to the bottom of the tube by the mere effect of gravity. Once there, only a small proportion of the molecules will occupy the volume of the test tube above the liquid. This fraction corresponds to the so-called equilibrium vapor pressure.

For instance, for water at 20 degree Celsius, the equilibrium vapor pressure p is 2.3 kilo-Pascal. For our purpose, the pressure is of little significance. One would rather wish to know the density ρ which corresponds to p. Fortunately, if one assumes that the vapor above the liquid can be described as an ideal gas (which is certainly true at such a low pressure) the two variables are related by the equation of state of an ideal gas which can be written:

$$p = \rho r_w T, \text{ where } T = \text{Kelvin temperature, } r_w = R/M_w$$

R is the gas constant: $R = 8.3$ kgm^{2}s^{-2}K^{-1} and M_w is the mass of one mole of water: $M_w = 18$g. Thus, $r_w = 462$ m^{2}s^{-2}K^{-1}. For the density one gets:

$$\rho = p/r_w T = 2300/(462 \times 293) = 0.017\text{kg/cubic-meter} = 17\text{mg/liter}$$

If we remember that the density of air in standard room conditions is 1,200mg/liter, we see that the density of water vapor is about 100 times smaller.

When the inter-molecular attraction is smaller the boiling point will be lower and the vapor pressure will be higher. For instance, at a temperature of 20 degree Celsius, acetone has an equilibrium vapor pressure of 22.8 kPa. In addition, $r_a = r_w/3.2$. As a result, the density of acetone vapor will be about 32 times higher than for water. In contrast, the ratio of the boiling temperatures of water and acetone is only: $100/56 = 1.8$.

Beetles

The difference in behavior encapsulated in the previous experiment needs to be studied in more detailed way in separate clustering experiments. Fig. 7 summarizes an experiment for beetles.
It should be noted that because these beetles prefer dark places the experiment must be done under fairly uniform light.

In the future, such experiments should be repeated with a larger number of beetles and possibly in a ring-shaped container in order to eliminate the edge effect.

[7] Because the velocity of the tube must be higher than the velocity of the molecules, instead of a test tube the experiment would require a steel cylinder! This is rather a thought experiment.

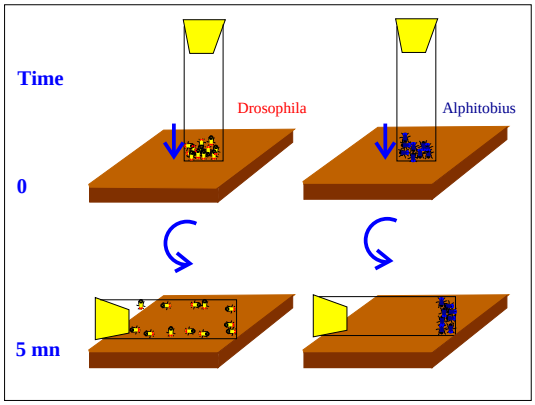

Fig. 6 Experiment demonstrating the existence of dispersion forces. This test-experiment which takes only a few minutes shows that there is a striking contrast between the behavior of drosophila and that of beetles (Alphitobius diaperinus). It could possibly be argued that the beetles do not move just because they are not active. In other words the fact that they remain together does not in itself prove the existence of attraction forces. However, it is difficult to explain the behavior of the drosophila without assuming the existence of agitation forces which are not kept in check by inter-individual attraction. *Source: Lai Shu Ying and Feng Meng Ying, Report for the "Championship in Experimental Science" at Beijing Normal University (Dec 2012).*

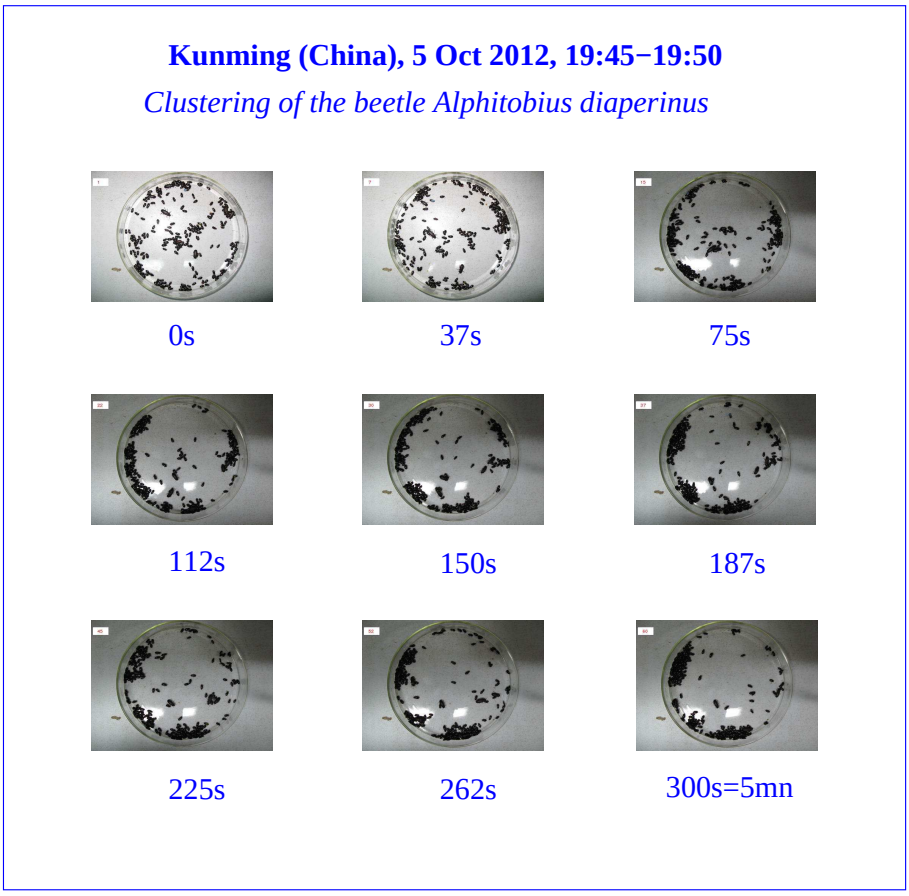

Fig. 7 Experiment showing the aggregation of beetles (Alphitobius diaperinus). The first action of the beetles is to rush toward the edge of the container. After this phase which lasts about 75s the positions of the beetles change only slightly. The diameter of the container was 15.5 cm. *Source: The experiment was performed on 5 October 2012 by Bertrand Roehner and Zhengwei Wang at the Eastern Bee Institute, Yunnan Agricultural University, Kunming, China.*

These beetles are the adult form of the so-called buffalo worms that are sold as food-stuff for birds or fishes. The transition to the adult form seems to be very dependent upon temperature and air humidity. At 20 C (and fairly dry air) it may take 3 months, whereas around 30 C it may take less than one month.

Drosophila

Drosophila are used extensively in genetics laboratories which makes them easily available. They have a natural tendency to climb upward. Thus, in the laboratory tubes in which they are kept, they are usually concentrated on the food which fills the bottom of the tube and at the top under the cap of the tube.

In the experiment described below, the Plexiglas containers had a height of only 6 mm which prevented from flying. We used low intensity, “cold” light which did not increase the temperature of the container. under these conditions we did not observe any clustering. Of course, it is quite possible that under different conditions an aggregation process may occur.

Part 3: Lessons and hints from physical chemistry

How to use aggregation/diffusion to measure interaction?

In this section we wish to examine what can be learned from physics about possible methods based on aggregation or diffusion for measuring interactions. This is a very natural step because physics has a long record of studying interactions between particles.

Aggregation

At first sight, aggregation phenomena may seem at odds with what one is used to observe in physics. Indeed, diffusion seems much more common than aggregation or clustering. For instance, how can one get an aggregation effect similar to the one described in Fig. 4a?
Consider a mixture of oil and balsamic vinegar. As the two liquids are not miscible and the vinegar has a higher density it will sit at the bottom of the bottle. Shaking the bottle will create a suspension of small, brown droplets of vinegar within the oil. However, within a few minutes the two liquids will separate again. This process is of course facilitated by the density difference but it also occurs without it as shown by the aggregation process of patches of oil floating on water or vinegar.

Aggregation will occur for systems characterized by a strong attraction and a low level of noise. In all other cases there will be diffusion, but the diffusion can be fast or slow, This leads to question the effect of inter-molecular attraction on diffusion?

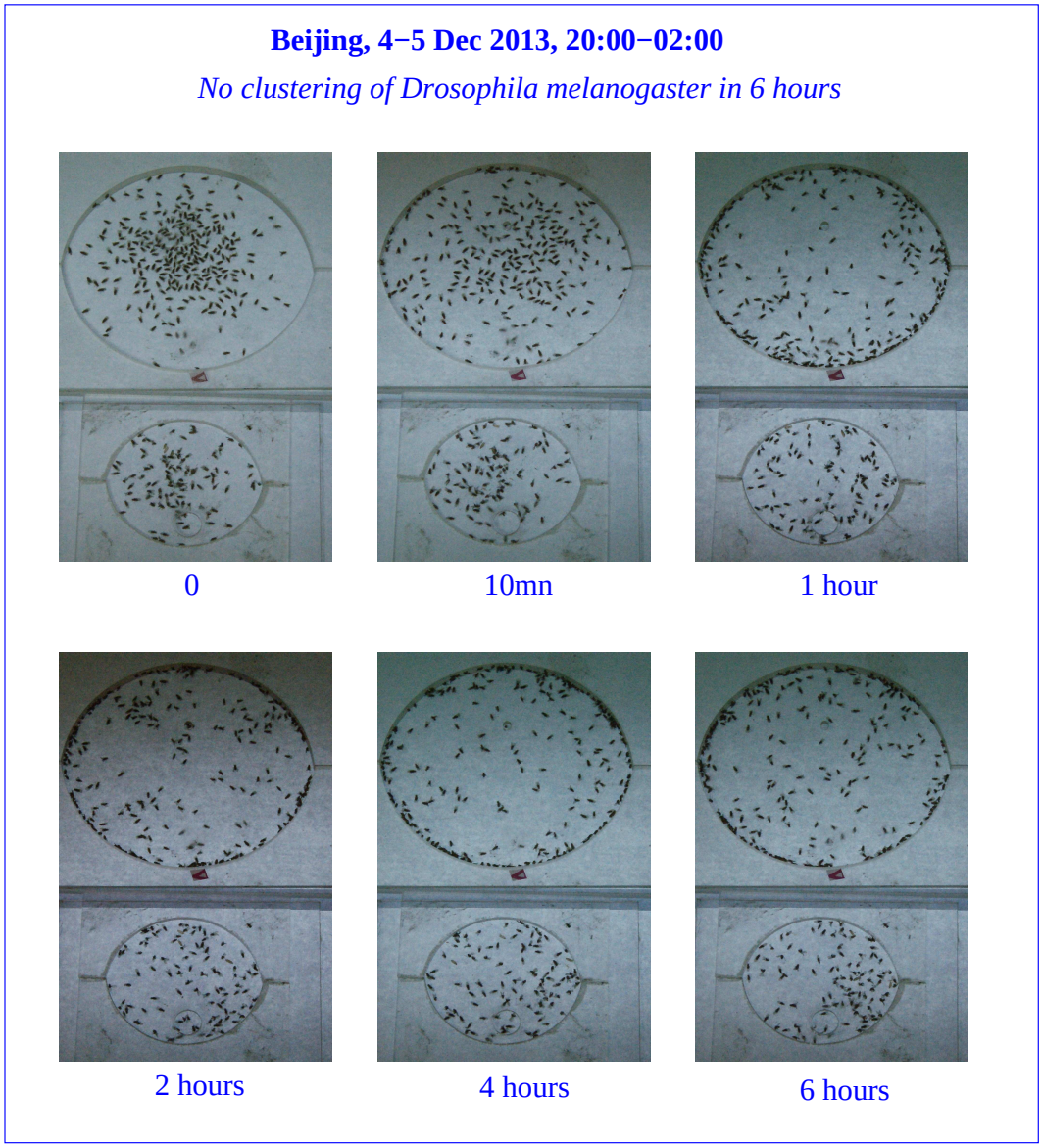

Fig. 8 Experiment showing that drosophila do not cluster together. In the lower cell there were 95 flies (density=247/sq-dm), whereas in the upper cell there were 244 flies (density= 309/sq-dm). At the end, after 6 hours, the density is fairly uniform in the lower cell; in the upper shell it is higher on the edge than in the middle but there is no clustering location along the edge. Of course, this does not mean that there are no interactions whatsoever among fruit flies. For instance, there is an obvious attraction between males and females. *Source: The experiment was done on 4-5 December 2013 at the School of Systems Science of Beijing Normal University. The fruit flies came from the genetics laboratory of Pr. Dou Wei (BNU). Many thanks to him.*

Can physics give us a method for deriving attraction strength from diffusion data?

Connection between diffusion and attraction

Surprisingly, when one tries key-words such as “diffusion”+“attraction” on an Internet search engine, one gets very few significant results. The mathematical theory of diffusion which leads to the partial differential equation known as the diffusion equation is purely based on random movements of *independent* particles. It describes well the diffusion of Brownian particles that is to say particles which are much bigger than

molecules. However, it is not appropriate for the diffusion of molecules A (e.g. dye molecules) among molecules B (e.g. water molecules) for in this case the $A-A$ and $A-B$ interactions play an essential role. This fact is made obvious by observing that depending upon these interactions, the A and B liquids will be completely miscible (e.g. water and ethanol), partially miscible, or almost not miscible (e.g. water and oil). Clearly, the degree of miscibility will affect the diffusion process.

Coming back to our previous Internet search, in order to improve the results, one should rather use the expressions "self-diffusion", also called "tracer diffusion", and "collective diffusion", also called "mutual diffusion"[8].
What physical phenomena do these expressions represent?

- Self-diffusion refers to the diffusion of individual molecules taking place in the absence of any concentration gradient; it probes the particle-particle correlation at small distance. In physical systems, self-diffusion simply reflects thermal molecular agitation. In systems of living organisms it reflects their movements. Such movements can also be seen as a form of thermal agitation because the chemical reactions on which motion relies will become slower if the temperature falls.
- Collective diffusion refers to the diffusion of a large number of particles (most often within a solvent) under the influence of a concentration gradient. This diffusion probes the correlations at large distances.

When there is no interaction between the particles, the diffusion coefficient is independent of particle concentration. On the contrary, for an attractive interaction between particles the diffusion coefficient tends to decrease as concentration increases. Thus, diffusion can be used to probe the strength of interactions.

When living organisms diffuse by swimming more or less randomly in water the only interaction that one needs to consider is their endogenous interaction. In other words, the physical analog that we need to consider is the diffusion of molecules A (the living organisms) among molecules B (the water molecules) when there is almost no $A-B$ interaction.

Two physical cases fulfill these conditions:

(1) Diffusion through evaporation of the molecules of liquid A in air.

(2) Diffusion of the molecules of liquid A in a liquid B with which they have no interaction. The lack of interaction means that the molecules cannot bind with one another which implies that the liquids are not miscible. However, even for two liquids which are not miscible, the mutual solubilities of A in B and B in A are not zero. For the case of water and liquid alkanes they are given in Table 2a,b along with the partial (equilibrium) vapor pressure in air.

[8] In this connection one can mention the following papers: Phillies (1974), Phillies et al. (1976), Van den Broek et al. (1981), Holmberg et al. (2011).

The two processes of evaporation and diffusion rely on a the same mechanism; it is schematically described in Fig. 9.

Table 2a Vapor pressure and solubility in water of liquid alkanes (at 20 degree C)

Name	Formula	Solubility of alkane in water [mg per liter of water]	Vapor pressure of alkane in air [kPa]	Density of alkane vapor in air [mg per liter]
n-pentane	C_5H_{12}	40	58	1715
n-hexane	C_6H_{14}	11	17	600
n-heptane	C_7H_{16}	2	5.3	217
n-octane	C_8H_{18}	1	1.5	70
n-nonane	C_9H_{20}	0.2	0.5	26

Notes: Vapor pressure (x) and solubility in water (y) decrease almost at the same rate. This suggests that the mechanisms of the two phenomena may be fairly similar. The $(\log x, \log y)$ correlation is 0.993 and the regression leads to the relationship:
solubility (mg/l) $= 0.5\,[\text{vapor pressure (kPa)}]^{1.1}$
This formula can be used to predict the solubility of decane ($n = 10$), undecane ($n = 11$), dodecane ($n = 12$). One gets (in micro-g/l): 66, 22, 3.8. As these solubilities are very low they are difficult to measure with acceptable accuracy. For decane 5 measurements performed by different authors range from 7 to 50 and their average is 32 micro-g/liter (Economou et al. 1997 p. 539).

The alkane solubility in water measures the diffusion of alkane molecules in water. Similarly, the density of the alkane vapor measures the diffusion of the alkane molecules in air. On average the alkane diffusion in air is about 80 times larger than its diffusion in water; more precisely the ratio increases from 43 in the case of pentane to 130 for nonane. These ratios certainly reflect the fact that it is easier for escaping alkane molecules to open their way in air than through water molecules. One is not surprised by the fact that this effect becomes stronger for bigger molecules.

Sources: Yang (2011, p.60). Wikipedia articles (in German). Comparison of data from different sources suggests that the accuracy of the solubility data is not better than 20%. The density of vapor was given by the ideal gas equation of state at 293K: $\rho = pM/RT = pM/2435$ *where* p *is the pressure and* M *the molar mass.*

Fig. 9 refers to the case of hexane but would be the same for any other liquid alkane. The data given in table 2a and the graph of Fig. 8 show that the diffusion has a clear correlation with the index n of the alkane. Moreover, one knows that the attraction between two alkane molecules increases with n because this attraction is due to a (weak) London-type attraction between the hydrogen atoms. Thus, the more hydrogen atoms there are, the stronger the attraction[9]. In other words, by measuring the diffusion rate is is possible to estimate the attraction strength, if not in an absolute way, at least in a relative way provided the comparison is made between species in which the interaction mechanism is the same.

From the perspective of applying the model of Fig. 9 to living organisms, it can be

[9]More details can be found in Roehner (2004, p. 100-103 and 2005 p. 663).

Table 2b Vapor pressure and solubility of water in liquid alkanes (at 20 degree C)

Name	Formula	Solubility of water in alkane [mg per liter of alkane]	Vapor pressure of water in air [kPa]	Density of water vapor in air [mg per liter of air]
Water	H_2O	50	2.3	7.4

Notes: The solubility data of water in liquid alkanes (from pentane to nonane) are almost independent of the alkane. Surprisingly, the density of water vapor in air is lower than the density of dissolved water in alkanes.
Sources: Encyclopaedia of hydrocarbons, Section 5.3: Treatment plants for oil production. p.644. Lucia et al. (2012, p. 10).
http://macro.lsu.edu/howto/solvents/pentane.htm (data for pentane at 20 degree)
http://macro.lsu.edu/howto/solvents/hexane.htm (data for hexane at 20 degree)
http://macro.lsu.edu/howto/solvents/heptane.htm (data for heptane at 25 degree)
The density of vapor at a given pressure was given by the ideal gas equation at 293K: $\rho = pM/RT = pM/2435$ *where* p *is the pressure and* M *the molar mass.*

observed that whereas the total number of layers is irrelevant in the case of molecules (because it is very large), it becomes an important parameter for living organisms. As long as the distance d between the external and deepest layer remains smaller than the range of the interaction ρ, the inward attraction on the external layer will increase along with the number of layers. This means that the diffusion rate will become smaller for a larger number of individuals. On the contrary, once the total number of individuals is large enough to make d larger than ρ, adding more individuals will not change the diffusion rate. This opens a way for estimating the interaction range, at least if the background noise is not too large.

How many "nearest neighbors?"

Let us assume that by some method based on diffusion or otherwise one has been able to estimate the interaction within a system of euglenas. From these global data one would like to derive an estimate of the interaction between two euglenas. How should this be done?
Once again physics can come to our help. The conclusion will be that, not surprisingly, the answer to this question completely depends on the number of neighbors with whom each euglena interacts[10]. This point will become clearer by examining a specific physical case.

Let us consider a liquid, for instance water or ethanol. A global estimate of its cohesion energy at molecular level is given by the heat of vaporization ΔH. It is

[10]In chemistry, that is to say inside a molecule, or in crystallography the number of nearest neighbors is called the *coordination number*. For a disordered system like a liquid, the coordination number cannot be precisely defined. Instead one will define successive coordination numbers for spherical shells of increasing radius.

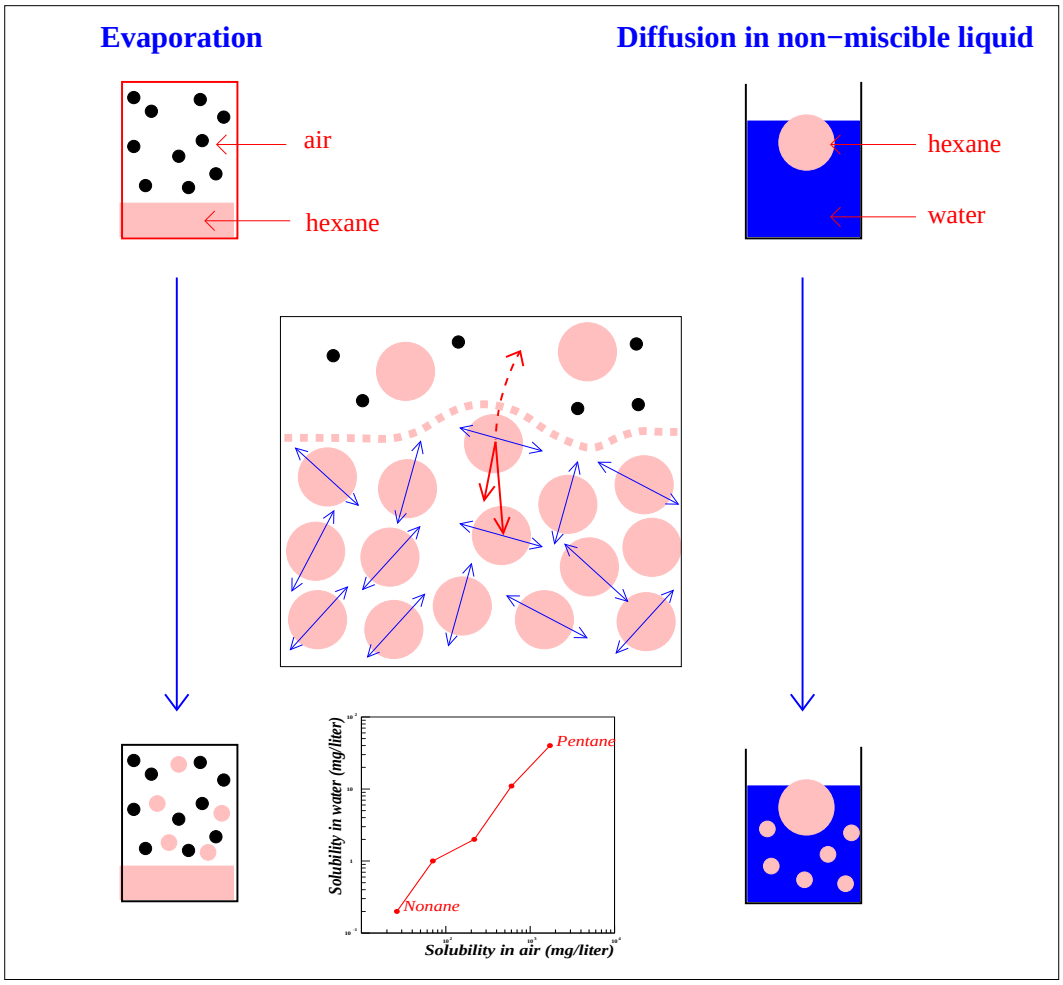

Fig. 9 Two similar diffusion mechanisms of a liquid A (hexane) into another fluid B. Left: Evaporation of A; this process can also be called "solubility in air". Right: Diffusion into a liquid B (water); this process can be called solubility of A in B (even though the two liquids are not really miscible). The two mechanisms have in common the feature that there is no interaction between the molecules which diffuse and the particles through which they diffuse. This similarity is exemplified by the graph which shows that from pentane to nonane the solubility rates decrease in the same way. The representation in the middle was drawn for evaporation but would be very similar for diffusion in water. The red arrows show the attraction forces experienced by an escaping molecule that is due to the molecules located in the first two layers. The blue arrows represent the thermal agitation of the molecules. It is this agitation which allows molecules in the external layer to escape from the liquid.

interesting to observe that, according to the so-called Trouton rule (see the Wikipedia article entitled "Règle de Trouton" (in French)) ΔH is, with good approximation, proportional to the boiling temperature expressed in degree Kelvin[11]:

$$\Delta H\big(\text{(expressed in kJ/mole)} = 0.087 T_b$$

[11] For liquids such as alcohols or water which have a H-bond the formula underestimates the vaporization heat. In those cases it should be replaced by: $\Delta H = 0.11 T_b$. However, if one is only interested in rough and quick estimates one does not need to take this correction into account. As a matter of fact, the formula gives acceptable orders of magnitude even when applied to gases or many solids. For instance:
For argon the heat of vaporization is 7.1 kJ/mol whereas $T_b = 87$ K gives 7.5.
For aluminum the heat of vaporization is 284 kJ/mol, whereas $T_b = 2743$ K gives 238.
But it does not work for sodium chloride whose heat of vaporization is 790 kJ/mol whereas $T_b = 1686$ K gives 146.

As an illustration we consider hexane whose boiling temperature is $T_b = 342$. The previous rule gives $\Delta H = 30$ kJ/mol, which is close to the experimental value of 31 kJ/mol.

For water we know both the global and molecule-to-molecule cohesion strength. The first one is given by $\Delta H = 40$ kJ/mol whereas the second one is 16 kJ/mol. The 16 kJ refer to a mole of pairs of water molecules. The ratio $40/16 = 2.5$ allows us to answer the question about the number of neighbors.

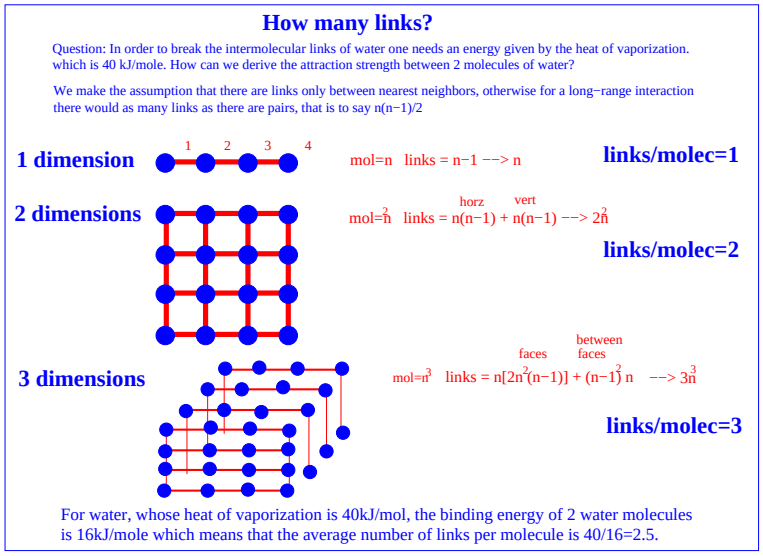

Fig. 10 How many links per molecule? The number of links per molecule depends upon the number of spatial dimensions and upon the specific structure of the system. In order to provide an illustrative case, this figure relies on the simplest possible assumption.

How should this result be interpreted? Fig. 10 considers the fairly ideal case of a rigid simple cubic structure in which, in addition, there are only interactions between nearest neighbors. In this special case, each molecule has 3 links. Naturally, this is a fairly unrealistic case. In a liquid, the structure is not rigid and the interaction decreases continuously with distance (as a power r^{-6} in the case of van der Waals forces).

In the case of hexane if we use the same ratio of 2.5 found earlier for water we get: $31/2.5 = 12$ kJ/mol for the binding energy between two molecules of liquid hexane. For heptane, octane and decane one gets similarly: 12.8, 13.6, 15.6. The fact that the interaction increases with the number of hydrogen atoms comes in confirmation of an argument made previously.

More generally we can write a general formula which gives the pair binding energy in any liquid (as well as gas or solid) as a function of its boiling temperature:

$$\text{molecule-molecule binding energy (in kJ/mole)} = 3.5 \left[\frac{T_b}{100}\right] \qquad (T_b \text{ in degree K})$$

The purpose of this formula is to provide orders of magnitude rather than accurate results. Its main drawback is that there is no real way to test the assumption that each molecule has an average of 2.5 “nearest neighbors”.

In conclusion, one should favor cohesion estimates at global level because particle-to-particle estimates must inevitably rely on shaky structural assumptions.

Sometimes, however, one needs estimates at pair level. An example is given in the next sub-section.

Gravity, noise and interaction in diffusion experiments

In a test-tube we first pour glycerol and then (very slowly to prevent mixing) water. This experiment raises two questions.

- Will there be diffusion despite the difference in density (the density of glycerol is 1.26)?
- Will the interaction between glycerol and water molecules play a role in the speed of diffusion?

Gravitation versus diffusion

To answer the first question we must compare the gravity potential energy of glycerol and the thermal agitation energy kT which brings about diffusion. In order to avoid small numbers we make the comparison for one mole which means that kT becomes $kN_aT = RT$ where R is the gas constant.
Assuming a height of 10 cm for the liquids, one gets:

$$E_p = Mgh = 92 \times 10^{-3} \times 9.81 \times 0.1 \sim 0.1 \text{ J/mol}, \quad RT = 8.3 \times 293 \sim 2.4 \text{ kJ/mol} \Rightarrow E_p \ll RT$$

Now that we know that there will be a diffusion effect, we can ask the key-question already examined above: will the diffusion be interaction-dependent?

Diffusion versus attraction

A quick answer can be obtained through a “continuity argument”. For two liquids whose molecules attract one another strongly (e.g. water-ethanol), there will be diffusion. On the contrary, for two liquids which have no interaction (e.g. water and oil) there will be no diffusion (except the tiny effect described above). Therefore, for intermediate cases it is natural to expect that diffusion will depend upon interaction strength.

The question can also be answered in a quantitative way by comparing kT, the thermal agitation energy of one molecule, and the interaction energy for a pair of molecules. The result given above in the previous subsection gives the interaction energy for water molecules, namely $E_{w-w} = 16$ kJ/mol. We do not know E_{g-g} or E_{w-g} but we do not really need to know them precisely. It is sufficient to know that

they are of the same magnitude as E_{w-w}. In short:

$$E_{\text{interaction}} \sim \frac{16/2}{2.4} \sim 4 \times (\text{Thermal energy})$$

In other words, at room temperature for a diffusion process involving two liquids A, B characterized by interaction energies simular to those of water[12]. the interaction strength plays a substantial role. However, whereas the ratio RT/E_p was a factor over one thousand, the ratio $RT/E_{\text{interaction}}$ is rather of the order of one.

There is a last point that should be added to the many lessons that physics can teach us. Although perhaps the most important, it is often overlooked.

The endless quest for ultimate details

It is often said that physical phenomena are "simpler" than biological or social phenomena.

No matter whether this is true or not, the success of physics relied on the fact that it first focused on overall understanding, leaving the intricate details of specific mechanisms for later investigation.

For instance, the detailed mechanisms of inter-molecular interactions are not only horrendously complicated but also of great diversity. As a matter of fact there are many, many types of interactions, e.g. ion-ion, ion-dipole, H bond, dipole-dipole, ion-induced dipole, dipole-induced dipole, London dispersion.

Moreover, each one of these interactions has many facets. Thus, for the H bond one may distinguish the following components: electrostatic attraction, polarization attraction, covalency attraction, dispersive attraction, electron repulsion.

Understanding any of these facets is in itself a formidable challenge both experimentally and theoretically. In addition, it is not obvious how scientifically "rewarding" gaining such an understanding may be.

Application to the case of living organisms

Two main conclusions emerge from the previous analysis.

(1) For strong attraction (ants, bees) there is clustering instead of diffusion. For low attraction (drosophila) diffusion leads to uniform density. Now, physical diffusion in the low solubility case suggests that for medium attraction one should expect a process in which diffusion stops before a uniform density is reached. Moreover, the equilibrium spatial density profile should give a measure of interaction.

(2) Until we have got a good quantitative knowledge of interaction strength for a broad range of species, one should not spend too much time on the investigation of

[12] Because the diffusion is also affected by viscosity one must also assume that the liquids have comparable viscosity.

detailed interaction mechanisms. The example of physics shows that such mechanisms are very complicated and highly diversified.

Part 4: In search of a general measurement method

Firstly, we focused on cases in which the attraction was either very strong (ants,bees, beetles) or very weak (drosophila). In this way, we were able to propose methods for estimating attraction strength. However, this left open the question for all cases in which the attraction is not strong enough to produce a clustering process.

In this part we set ourselves a challenge. We consider a specific species of microorganisms, namely *Euglena gracilis* for which we wish to measure the interaction strength. As we do not know in advance whether the interaction is strong, weak or nonexistent this is a much more difficult task than what we have done in the first part. It is an ongoing investigation. We will report it chronologically highlighting unexpected difficulties. We made some progress but more work is still required in order to reach our objectives.

Euglena gracilis

Euglena gracilis is a unicellular organism which swims in water. When moving it has the shape of a cylinder with a length of about 50 micrometers and a diameter of about 10 micrometers. Its velocity is of the order of 1, 2 or even 3 times its length per second. It is of green color because it contains chlorophyll.

Why did we select it? There were practical reasons as well as scientific reasons.

- The euglenas are easy to keep. A population kept in a small bottle for over one month will remain in good shape. After two months many cells will take on a circular shape and become fairly static. This indicates that there is need for a “revitalizing” process which requires special expertise, specific chemical products and techniques.
- Due to their small size, a drop of euglenas will contain thousands of individuals which is a favorable factor for the investigation of collective phenomenon. On the other hand, their size is large enough to make them observable with a stereo-microscope[13].
- *Euglena gracilis* has been studied and used as a model microorganism at least since 1880. The references of some early papers and books, e.g. Engelmann (1879, 1882), Stahl (1880), Mast (1911), can be found in the reference section.

[13]Stereo-microscopes always have two eyepieces. Since stereoscopic vision requires two distinct images, one presented to the left eye, and one to the right, they must have two objectives. High power microscopes also have two eyepieces but only a *single* objective lens. Because of their small magnification (typically between 20x and 80x) stereo-microscopes have a broad observation field which is an important requirement for our observations.

Research plan

When we started our research in December 2013 our plan was to proceed in three steps.

(1) First, we wanted to measure the main individual characteristics, such as sizes, average and standard deviation of the velocity distribution of the euglenas.

(2) Secondly, we wanted to see if we could identify expected effects such as the "crowded sidewalk" effect .

(3) Last but not least, we wanted to find a method for measuring the interaction strength. This is the most important but also the most challenging part. In accordance with the physics-based argument given above, our plan was to try a method based on the diffusion properties of the euglenas. It is at this point that several surprising observations made us realize that we had underestimated the sensitivity of euglenas to low-intensity light. Since, needless to say, it is impossible to make observation in total darkness, we realized that it was necessary to study the effect of light in order to find out what level of light was acceptable.

Among the surprise-observations that made us change our program, one can particularly mention the following.

(1) Euglenas were found to flee light even at low intensity, e.g. 200 lux, some 10 to 100 times less than what we expected from the literature.

(2) The diffusion behavior of euglenas is completely changed even under low intensity light. Basically the diffusion is stopped.

(3) A sudden change in the intensity or color of light "freezes" the euglenas, a reaction referred to in the literature by the German expression *Schreckbewegung* (shock reaction).

An account of these observations is given below.

Fig. 11 proposes a recapitulation of the range of questions that arise when one wishes to study the properties of a system of living organisms.

The classification takes its inspiration from physics; it provides a quick assessment of the expected difficulty of a question in the sense that:

- Time-dependent phenomena are more difficult to study than equilibrium situations.
- Collective properties are more difficult to study than individual properties.

Individual properties

As an introduction we wish to propose a riddle and to show a film.

Positions and orientations

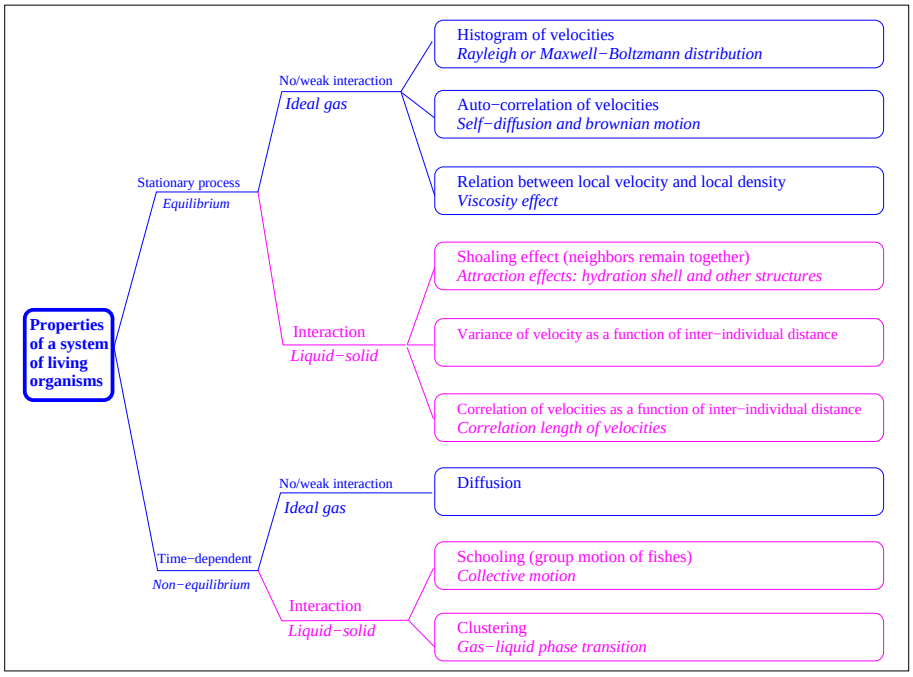

Fig. 11 Properties of a system of living organisms. The questions are arranged in the form of a tree which is modeled on the classes of questions considered in statistical physics. These questions are written in italic.

Fig. 12 has 4 panels. Two of them have been drawn according to real pictures of euglenas. All little segments have been drawn in the positions and orientations of the euglenas on the pictures. On the two other panels the positions and orientations were selected randomly. For x and y the numbers were drawn from a uniform distribution on the interval $(0, 1)$, and the angular position was drawn from a uniform distribution on $(0, 2\pi)$.

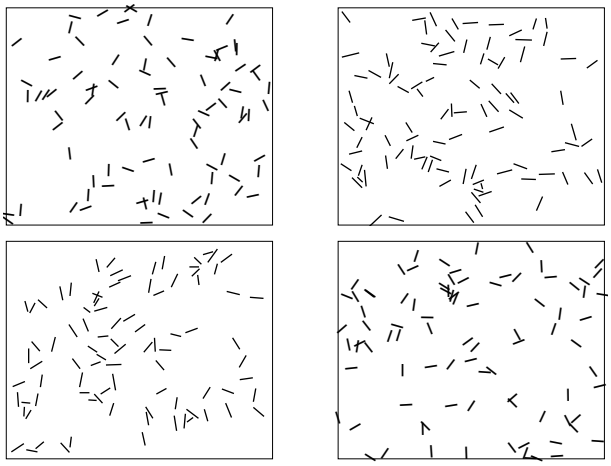

Fig. 12 Simulations versus real pictures. Two of the images are simulations whereas the two other reproduce the positions and orientations of real pictures. Is it possible to distinguish between them?

Is there a way to distinguish the real pictures from the simulations? We leave the answer to the reader but personally we were not able to find a clear criterion[14].

[14]The pictures of the real euglenas are 1 (top left) and 4.

Positions and orientations give only a static picture. By watching a movie[15] we will get a dynamic picture.

It reveals some interesting points.

- Euglenas may take two aspects: cylindrical segment-shaped or disk-shaped. In the second the euglenas do not move, except perhaps rotating on a vertical axis.
- Some euglenas move straight ahead. Clearly such deterministic movements are very different from the erratic zigzags of a Brownian particle. In other words, whereas the positions and orientations of the euglenas were consistent with a purely random model, their movements are not.

A more quantitative analysis will be to draw the distribution of velocities.

Distribution of sizes

Before that, however, there is another static property that we need to examine, namely the distribution of sizes. The distribution of sizes will affect the distribution of velocities and if it is fairly broad it will be important to measure the velocity distribution on samples restricted to a given size. Fortunately this is not necessary because the distribution of size is fairly narrow. It has a coefficient of variation (i.e. standard deviation/average) of 0.11 which is much less than the coefficient of variation of the velocities.

Distribution of velocities

The Maxwell-Boltzmann distribution of molecular velocities in an ideal gas is well known by physicists. It can be derived theoretically in the framework of the kinetic theory of gases. Less well known, however, are the experimental tests of this law. Direct experimental measurements did not come before the 1930s. and their accuracy remained hardly better than 5%.

The pioneer experiment was the observation of the effusion effect, that is to say the production of a molecular beam, by Louis Dunoyer (de Segonzac) in 1911. However, its experiment remained more qualitative than quantitative. The first experiments that was accurate enough to allow a comparison with the M-B distribution came almost 20 years later first by Lammert (1929) followed with a better accuracy by Zartman (1931). Zartman's results are in good agreement with the M-B distribution for high velocities but not for the lowest velocities.

A point of particular interest for the present experiments would be to know whether or not the distribution is changed when the source is a liquid instead of a gas. A paper by Johnston et al. (1966) provides a comparison for the case of helium II but because many corrections had to be performed the interpretation of the results remains fairly

[15] Available at the following address: http://www.lpthe.jussieu.fr/∼roehner/euglena.html

unclear.

For the experiment with euglenas we took several movies each of which had 500 images and corresponded to an observation time of 77s. The observation field measured $2.02\text{mm} \times 1.52\text{mm}$. The euglenas were moving between microscope slide and cover slip and the movie shows that they could not cross one another. In other words it was a two-dimensional experiment.

For the highest density (18/sq-mm) the observation field contained about 80 euglenas but some 25% of them were disk-shaped euglenas which did not move[16]. All the cylinder-shaped euglenas were followed over 10 images (1.54s). The distance covered was approximated by a straight line from initial to final point. Then, the same operation was repeated for a second series of 10 frames located about 10 seconds later so that it may be considered as a realization independent of the first one. This procedure was iterated several times.

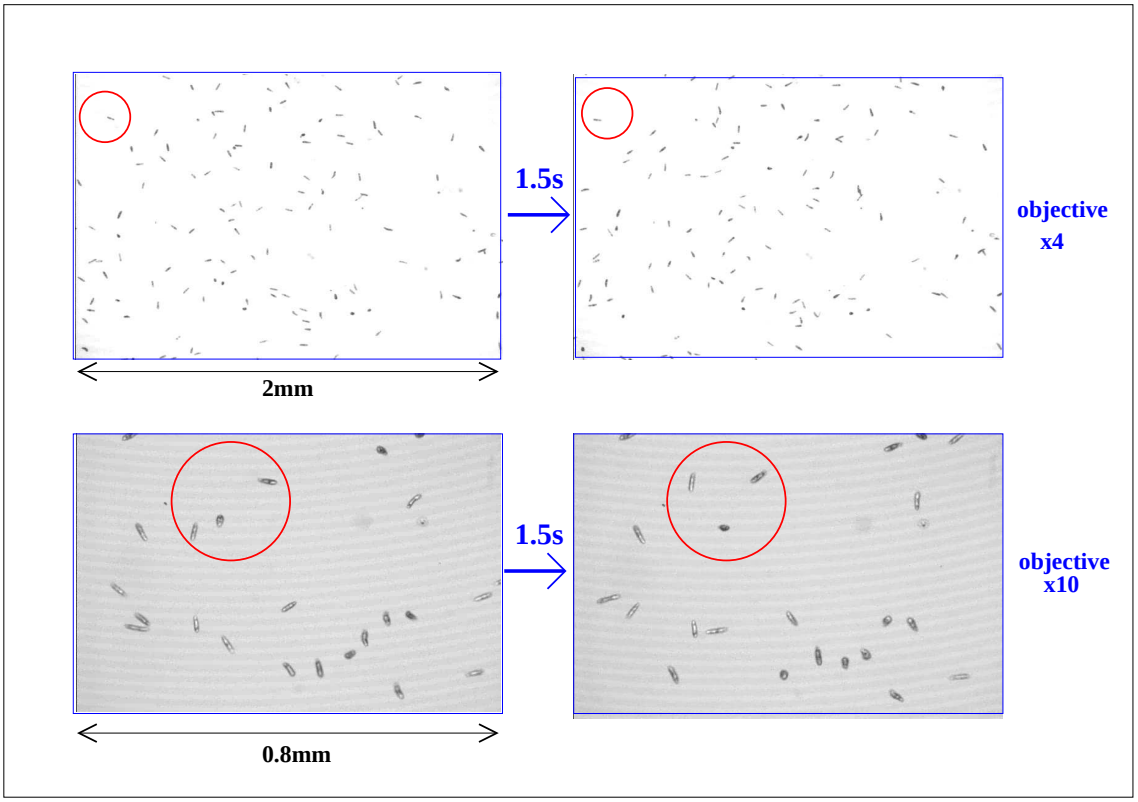

Fig. 13a Pictures of euglenas over a time interval of 1.5 second. The two pictures in the first line show the sort of pictures used for measuring the distribution of velocities. The red circles identify the movement of one specific euglena. The pictures in the second line provide a better view of the shape of the euglenas but for this higher magnification the number of euglenas contained in the observation field is too small for the purpose of measuring velocity distributions. *Source: The observations were performed in December 2013 in the "Cell cycle and cell determination" group of University Pierre and Marie Curie.*

[16]Only a few euglenas switched from disk-shape to segment-shape or vice versa during the 1.54s time intervals used for measuring the velocities. Therefore this effect was neglected.

Prior to analyzing the distribution of velocities, there was a first useful check to be made which was the following. Needless to say, to take the movie the sample had to receive low intensity of light coming from below the sample. In order to check whether or not the light had an influence on the motions of the euglenas (for instance due to non-uniformity) we computed the sum of the velocities of all euglenas on a given frame. A non-zero sum would reveal a drift due to an asymmetry of the light-source. In fact, the averages of the x- and y-components were less than 5% of the average distance covered over the 10 frames. Nevertheless a correction was performed by subtracting the drift before drawing the velocity distribution.

Before studying the modulus of the velocities, we studied their x- and y-components. Their distributions turned out to be very well approximated by a Gaussian distribution. The test was performed through the so-called "Henry method" (see the Wikipedia article in French entitled "Droite de Henry"). It is based on the fact that the values of the inverse function of the cumulated normal distribution as a function of the values of the random variable should be a straight line if the distribution is Gaussian. It was indeed a straight line with a coefficient of linear correlation equal to 0.995. What will be the implication of this result for the distribution of the modulus of the velocity?

In probability theory there is a theorem (see for instance Parzen 1960, p. 324) which says that if $X_1, X_2, \ldots, X_n$ are independent random variables each normally distributed with mean 0 and same variance, then $Y = \sqrt{X_1^2 + X_2^2 + \ldots + X_n^2}$ follows a χ distribution.

Moreover, from the properties of the χ distribution[17] one knows that its coefficient of variation is given by the following formulas[18]:

$$CV_{n=1} = \sqrt{\pi/2 - 1} \simeq 0.75, \quad CV_{n=2} = \sqrt{4/\pi - 1} \simeq 0.52, \quad CV_{n=3} = \sqrt{3\pi/8 - 1} \simeq 0.42$$

- The case $n = 1$ would correspond to euglenas moving in a narrow capillarity tube with a diameter the size of the euglenas.
- The case $n = 2$ corresponds to euglenas moving between slide and cover slip as in our observation. In this special case the χ distribution is called a Rayleigh distribution.
- The case $n = 3$ corresponds to 3-dimensional movements; it can be either euglenas or the molecules of a gas. In this special case the χ distribution is called a Maxwell-Boltzmann distribution.

From the results shown in Fig. 13 it appears that the coefficient of variation is not

[17] See for instance the Wikipedia article entitled "Chi distribution"

[18] The fact that the coefficient of variation does does not depend upon the variance of the Gaussian variables is not really surprising. It is due to the positivity of Y; as a result, an increase in the variance will also increase the average. Moreover, it can also be shown that when $n \to \infty$, the coefficient of variation converges toward 0: CV $\sim 0.7/\sqrt{n}$.

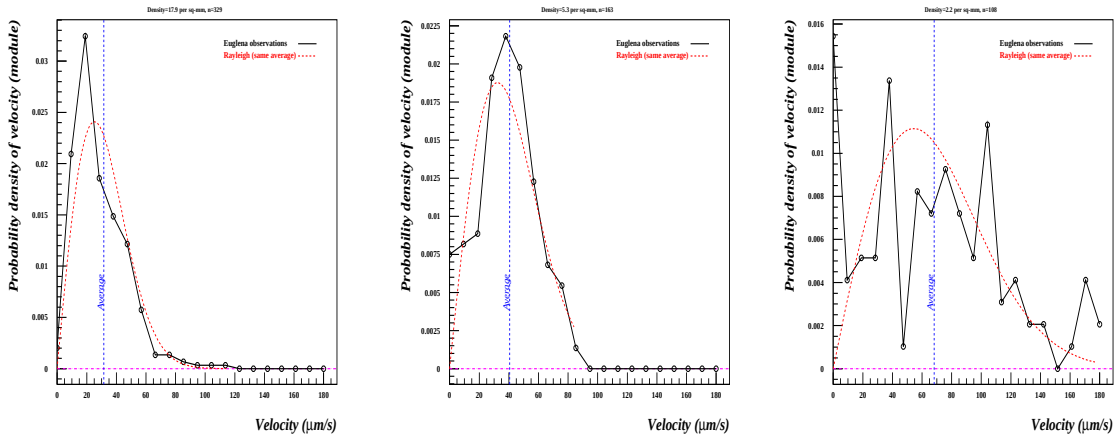

Fig. 13b Velocity density distribution of euglenas. The 3 graphs from left to right correspond to decreasing densities: 18/sq-mm, 5.3/sq-mm, 2.2/sq-mm. To these decreasing densities correspond increasing average velocities: 31 micro-m/s, 40 micro-m/s, 68 micro-m/s. The coefficient of variation does not show any clear trend. Its average for the 3 cases is 0.55, not far from the coefficient of variation of a Rayleigh distribution which is $CV = \sqrt{4/\pi - 1} = 0.52$. *Source: The observations were performed in December 2013 in the "Cell cycle and cell determination" group of University Pierre and Marie Curie.*

far from the value 0.52 corresponding to the Rayleigh distribution. One can also see that, in accordance with what is expected from the crowded sidewalk effect, the mean speed decreases when the density increases .

Velocity distribution for ants

A similar study based on data for fire ants published in a book by Pr. Deborah Gordon (2010, fig. 3.1) led to a coefficient of variation of 0.55.

Temperature effects on movement

It is well known that the average speed v_m of the molecules of a gas increases with Kelvin temperature T: $v_m \sim \sqrt{T}$. It would not be surprising to observe a similar effect for living organisms. For instance it is well known that the activity of ants or bees is fairly low when the temperature is below 10 degree Celsius. Such an effect has also been observed for groups of small fishes by students at Beijing Normal University.

A similar study for bacteria can be found in Schneider and Doetsch (1977, p. 697). They studied 4 species of bacteria[19] over a temperature range from 10 to 40 degree Celsius. For each species, the average speed increased with the Celsius temperature t like a function of the form $t^\alpha,\ \alpha > 1$. Over the whole range the velocity (averaged over the 4 species) was multiplied by 6.1. Such an increase is of course much faster than the increase of $\sqrt{T}$.

[19] *P. mirabilis, S. typhimurium, S. serpens, P. fluorescens*

Relationship between local density and speed

In the previous section we have seen that for *whole samples* of different densities the average speed decreases when the density increases. In Fig. 12 we have also seen that within a given sample the density is not uniform. This leads us to the question of whether it is possible to identify a connection between local density changes and changes in spatial average of velocity.

Methodology

In its principle the methodology is straightforward. We divide each image into a number (p) of zones, then we compute the averages of both the density (d_m) and the velocity (v_m) in each zone; finally, we test the correlation of the (d_m, v_m) points. The main difficulty is to implement this procedure in a way which minimizes the background noise.

More specifically, we divided each image into $3 \times 2 = 6$ zones[20]. Why 6 zones? It appears to be the best compromise between two conditions which can be explained as follows. In order to minimize the fluctuations of the averages, one needs as many euglenas as possible in each zone. This implies big zones. On the other hand however, each zone (over the time interval required to compute the velocities) will give only one (d_m, v_m) data point. Thus, big zones will give few data points and in addition they will have a fairly narrow dispersion. This, in turn, will lead to large confidence intervals for the correlations. On the contrary, small zones will give a larger number of fairly "noisy" data points. In order to find the best compromise we tried several subdivisions. The division into 6 zones turned out to be the best choice. With this choice there were about 10 euglenas in each zone.

Results

Altogether, based on 62 (d_m, v_m) data points, one gets the following correlation between density and velocity:

density-velocity correlation: $r = -0.55$, confidence interval (0.95 confidence level) $= (-0.7, -0.2)$

As zero is outside the confidence interval the correlation can be said to be significant.

Is the (density,velocity) connection a self-reinforcing mechanism?

The previous correlation implies that when an euglena arrives in a high density zone it will slow down. As a result, by spending in this zone more time than elsewhere, it will contribute to the crowding. If this is really a self-reinforcing mechanism it should result in spatial density disparities that are sharper than those which result from the mere effect of randomness.

In order to probe this effect one would need a series of pictures (taken almost at

[20] The images have 696 pixels in the x direction and 520 in the y direction.

the same moment) covering a large area of the sample. Why can we *not* use frames (sufficiently apart in time) of a film focused just on one narrow area? The reason can be understood by taking a look at the figures given above for the clustering process for ants. In this case the self-reinforcing mechanism is made obvious when we watch the whole population because we see a number of clustering centers. However, if one restricts the observation to a narrow area far from the clustering centers it is likely that the density pattern will not be very different from one based on purely random numbers.

The observation of a broad spatial area will be one of our objectives in forthcoming experiments. To begin with, this can be done in a capillarity tube. However, one needs to be very careful about possible disruptive effects of light. The perturbations brought about by light are described in the next section.

Reactions brought about by light

We describe 4 experiments which show various reactions of the euglenas to light or lack of it.

Positive versus negative phototaxis

Phototaxis is the ability to move in response to light. The broad rule is that euglenas are attracted by weak light and on the contrary repulsed by strong light. However, it seems that there is no clear agreement in the existing literature regarding the threshold between the two types of behavior. This may be due to the fact that the reaction of the euglenas to some extent depends upon their previous exposure. Although the objective of the experiment described in Fig. 14 was more qualitative than quantitative it sets the boundary between the two responses somewhere around 100 lux[21].
It can be noticed that the gathering of the euglenas in the positive phototaxis experiment was about three or four times slower than in the negative phototaxis experiment.

No diffusion in dim light versus diffusion in darkness

When a batch of euglenas is introduced into a capillarity glass tube (1.15mm in diameter) their behavior is very different depending on whether the tube is put under uniform dim light or in complete darkness. In the first case, there is almost no diffusion, whereas in darkness the euglenas spread to the whole tube within 30mn approximately.

This is illustrated in Fig. 15. The first three panels concern the case with light. In the last panel the green curves show the frozen diffusion under dim light whereas the

[21] 1 lux corresponds to a full moon night, 150 lux is what is needed to read, 130,000 lux corresponds to sunlight on a bright summer day. The last case corresponds to 1000 W/sq.m which gives an equivalence between the two units valid for sunlight.

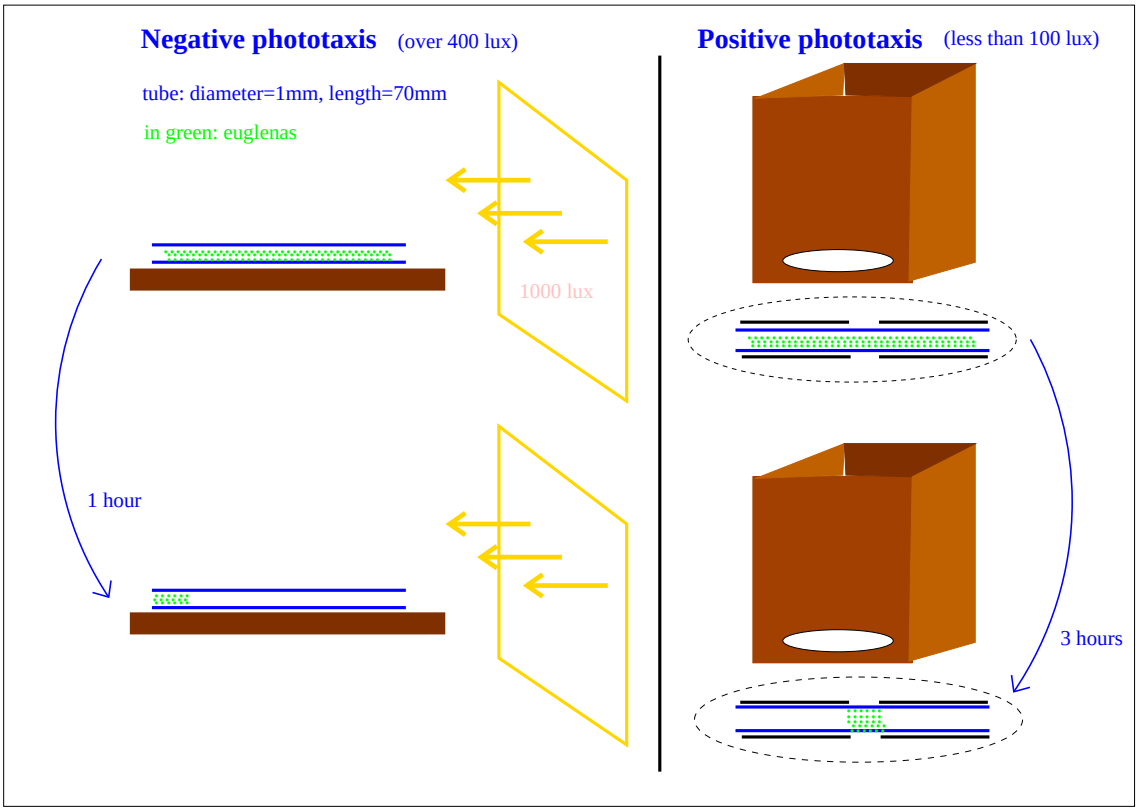

Fig. 14 Positive versus negative phototaxis. The tube containing the euglenas have an internal diameter of about 1mm. In the experiment on the right-hand side a sheath recovered the tube except for a 5mm interval around the middle of the tube which could receive the faint light that could reach the bottom of the box. *Source: The observations were performed in March-April 2014 at the LPTHE (University Pierre and Marie Curie).*

black curves show fast diffusion in darkness.

So far we do not have any explanation for the experiment described here. It can hardly be explained in term of phototaxis because it is characterized by a lack of motion.

Another behavior described by early observers (Engelmann 1882, Mast 1911) consists in the fact that a light spot acts as a trap for the euglenas: once they have entered it they cannot leave it (like a night butterfly which flies around a lamp). However this does not apply here for there was fairly uniform, low intensity light on the entire capillarity tube.

One question remains unclear: was the light level really uniform? In other words, was the luminance the same in all directions? In appearance it seemed so. However, the fact that we used two fibre-optic light sources, one on each side, raises some questions. Although these sources were at least 10cm distant from the sample it is possible that there were two opposite light gradients. If this is the right explanation for the frozen diffusion, and as these gradients were certainly fairly small, it shows that the euglenas are sensitive even to slight light gradients.
In the future the experiment will be repeated in different conditions.

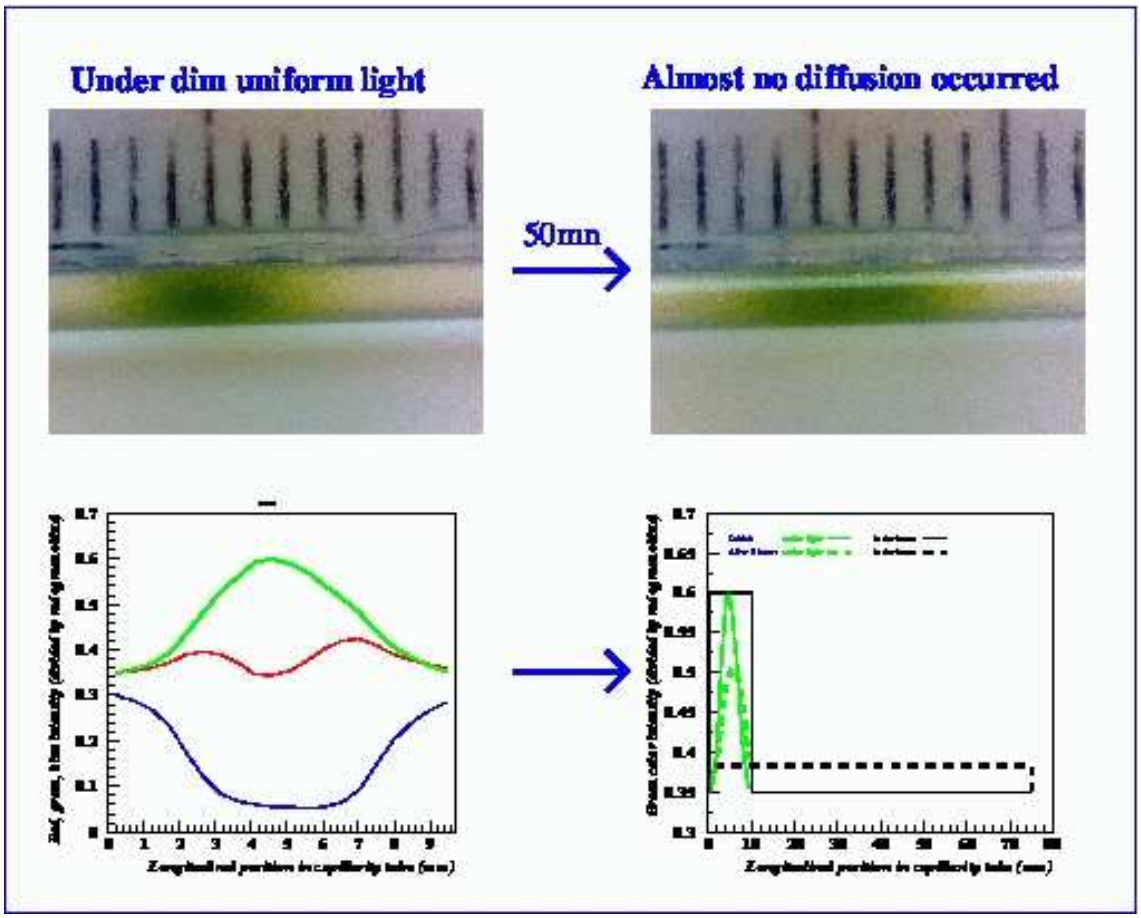

Fig. 15 Negligible diffusion under dim light versus quick diffusion in darkness. The two top panels show that under dim light there was almost no change in the concentration of the euglenas. A quantitative confirmation was obtained by analyzing the longitudinal distribution of the green color using software commands from ImageMagick. Panel 3 shows the spatial distribution of red, green and blue light at the start of the experiment. The last panel shows that after 50mn the concentration profile (measured by the green color) is almost the same as at the beginning. The internal diameter of the capillarity tube was 1.15mm and its length was 7cm. The light provided by the microscope had a fairly uniform intensity throughout the tube length.
On the contrary, in complete darkness there was a marked diffusion process with the result that after about 30mn the euglenas occupied the whole length of the tube. This is represented by the black curves: the solid line is for the beginning of the experiment whereas the low dotted line represents the fairly uniform distribution after 30mn. These curves are rather schematic because we avoided light as much as possible. *Source: The observations were performed on 31 March and 1 April 2014 at University Pierre and Marie Curie.*

Effect of a sudden change in light intensity: *Schreckbewegung*

When the light is suddenly changed from red to blue the euglenas stop moving, take on a circular shape (perhaps trying to minimize their exposed area). This occurs very quickly in less than 0.3 s. As this behavior was first discovered and described by German researchers it became known as *Schreckbewegung* which means shock reaction. It can be noted that as the energy of red light is about one half of the energy of blue light[22] a switch from blue to red amounts to a reduction in light intensity. Moreover the euglenas may also be more sensible to some wavelength intervals than

[22] $E = h/\lambda$, where h is the Heisenberg constant and λ the light wavelength.

to others.

Light induced clustering

Fig. 16 describes two experiments in which the clustering of the euglenas is brought about by their exposure to strong light. What are the similarities and differences between the two experiments?

- In both experiments the light comes from below. Another similarity is the reaction time which is of the order of 5mn.
- In the second experiment the height of the container is about 20 times smaller than in the first.
- Another difference is that in the second experiment the light is applied only to a fraction of the container area, the rest of it receiving whatever light comes (through diffusion and reflection) from the lighted zone.

When the light is turned on the euglenas in the lighted zone have a reaction similar to the *Schreckbewegung* described previously. Yet, the cluster does not appear in this zone but in its darker neighborhood. The experiment was repeated several times with same results.

Evidence of interaction

There are two fairly different methods for the identification of interactions between the euglenas.

- The first method is to analyze a series of pictures taken at sufficiently short time intervals to allow a measurement of instantaneous velocities. The hope is that the movements of the euglenas will reveal some kinds of pattern which are typical of interactive individuals, For instance, if the correlation between their velocities decreases for larger separation between them one might be think that it is a result of their interaction. However, it can also be a consequence of the side-walk effect. Indeed, if the euglenes slow down in areas of higher density this will result in a separation-dependent correlation.
- The second method is to observe a form of collective motion that cannot easily be explained without interaction.

We have tried the two methods. In the films in which the euglenas were between slide and cover slip we never saw any clear. pattern of collective behavior. It was probably due to the fact that there was only one layer of euglenas. Another factor is the fact that when working with a substantial magnification the observation field is so small that one may well miss an existing collective pattern.
One of the clearest results obtained in analyzing the movements was the relationship between average velocity and local population density. The other tests were rather

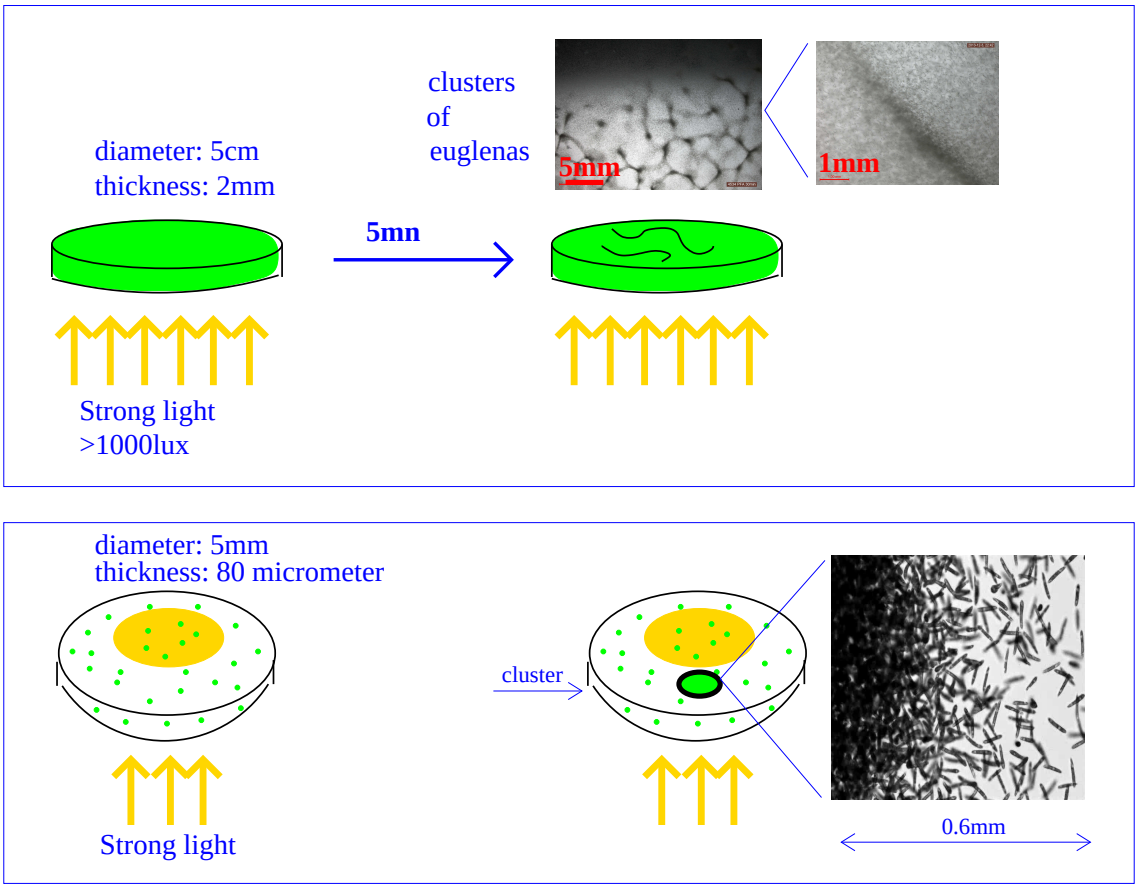

Fig. 16 Clustering brought about by exposure to strong light. In both cases the clustering process takes about 5mn which means that it is some ten times faster than the clustering of ants described previously. *Source: The first observation was analyzed in detail in Suematsu (2011). The pictures were taken in December 2013 and March 2014 at Beijing Normal University and University Pierre and Marie Curie respectively.*

inconclusive.

In the two following subsections we describe two collective movements: (i) the formation of clusters (ii) the formation of networks.

Aggregation of euglenas

As already said, the formation of high density areas can be explained by the side-walk effect. However, the clusters shown in the pictures are extreme forms of high density areas in the sense that the euglenas form a solid bundle in which they seem to be completely steady; in fact the bundle is so compact that it is difficult to see what happens inside. The main point is that without any attraction, the euglenas would just swim around the clusters whereas on the contrary one sees a permanent flow of euglenas entering and leaving the cluster.. In other words, one has the feeling that such clusters cannot be explained through the side-walk effect.

There are two other mechanisms that are mentioned in the literature for explaining the formation of clusters: one is self-shading and the other bioconvection.

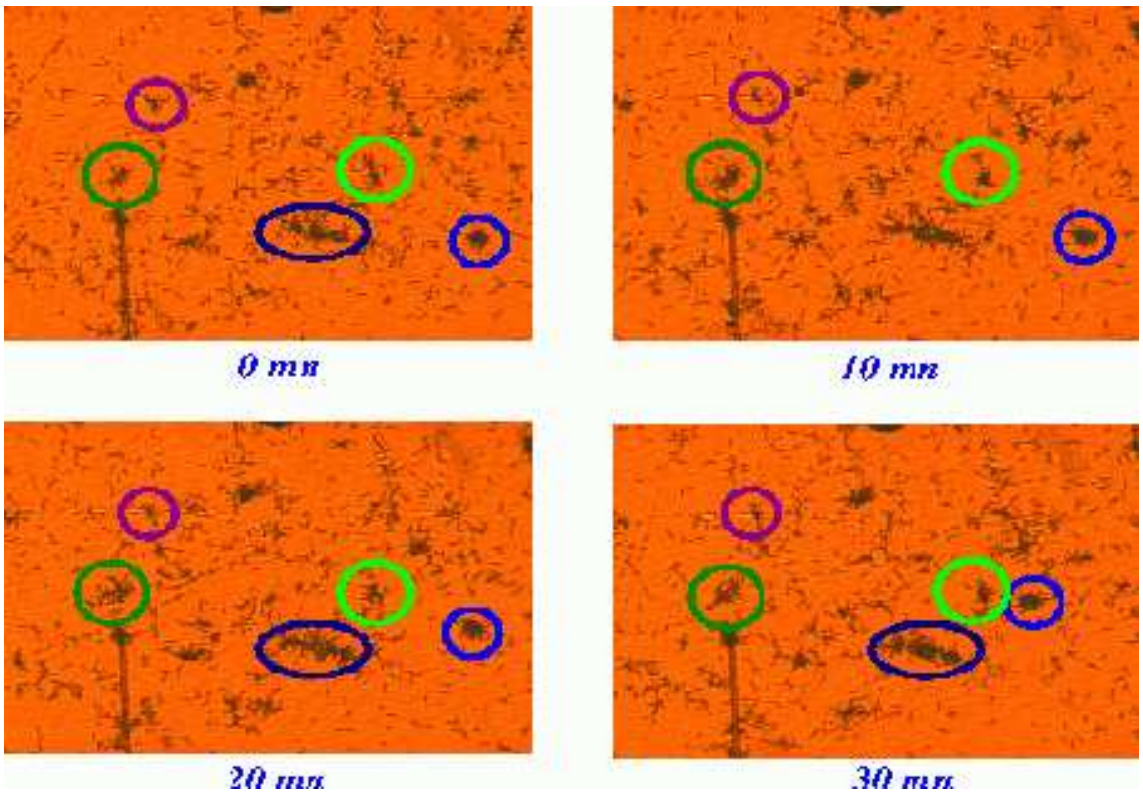

Fig. 17 Small- and medium-sized clusters of euglenas. When euglenas are observed between slide and cover slip there is only enough space for one layer of a thickness around 70 micro-meters; in such conditions they cannot cross one another and (for some unknown reason) do not form any stable clusters. In the present experiment the euglenas were *not* between slide and slip. They were in a kind of mini-swimming pool which was 2mm wide, 100mm long and 0.4mm deep. The pool had been made watertight to prevent evaporation. For most of the time the euglenas received very little light (less than 30 lux) except during the short time intervals when pictures were taken. During these moments the light came from above for it was an inverted microcope that was used. The circles and ellipses show clusters of various sizes. The cluster inside the light blue circle can be seen to move around globally. Also of interest is the fact that the mini-cluster inside of the magenta circle almost disappeared. *Source: The experiment was done on 14 May 2014 at the University Pierre and Marie Curie (IMPMC)*

The conditions of the present experiment were chosen with the purpose of eliminating these effects.

To eliminate self-shading the euglenas were left all the time in low light (less than 30 lux) except when the pictures were taken.

To eliminate bioconvection that is to say vertical up and down movements of the euglenas we put them in a swimming pool whose depth was only 0.5mm. This would allow several layers of euglenas but would likely be too narrow to have any substantial vertical movements. In addition, whereas bioconvection experiments involve a bright light source beneath the euglenas, in our experiment there was none.

Network formation

Observation

Observation shows show that, in some conditions, the euglenas form a kind of lattice.

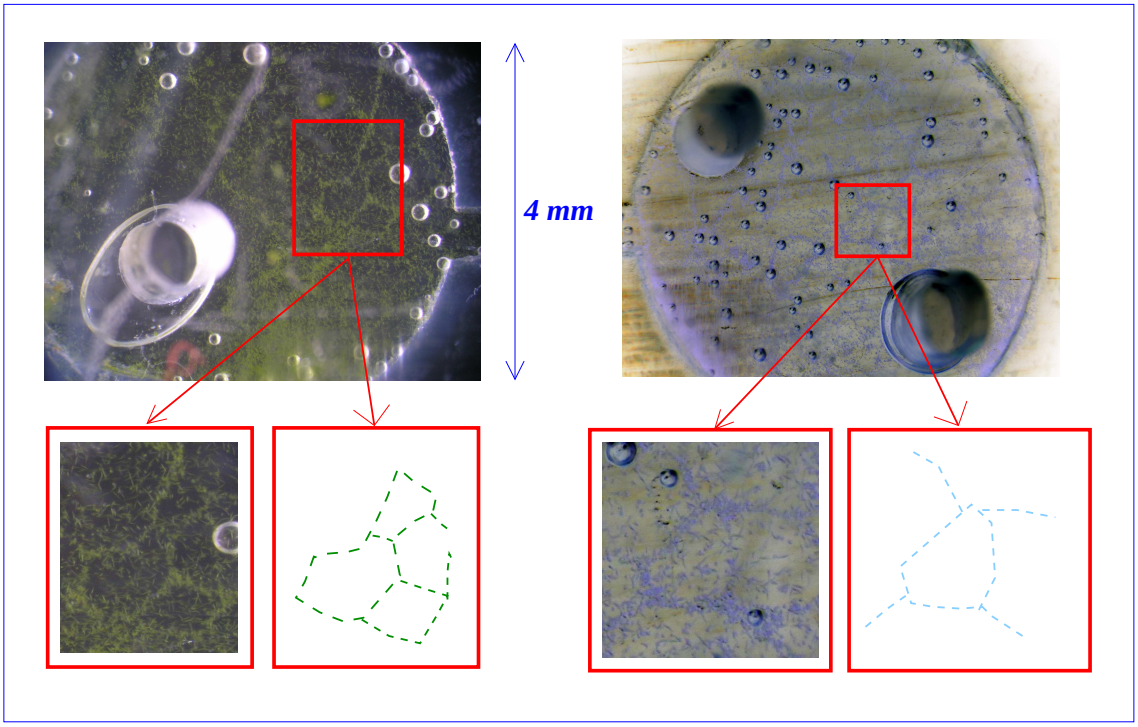

Fig. 18a Formation of networks of euglenas. The "swimming pool" of the euglenas comprised two circle-areas connected by a narrow canal. However, in the present formation of networks the communication canal probably did not play any role for each circle-area seemed to change independently from the other. The depth of the pool was 0.5mm which means that there were certainly several layers of euglenas. The network appeared some 20mn after the device had been filled (at 11:15 am). It subsisted almost unchanged for several hours but disappeared during the night. At 9:00 am on the following morning all euglenas were on one side in the left-hand side circle-area. In this area their density was fairly uniform. The image on the right-hand side is a negative version of the original picture; moving over to the negative slightly improves the contrast. The origin of the small bubbles is unclear. They disappeared a few hours later. The 3 big circles (one on the left and two on the right-hand side) are the holes (of diameter 1mm) through which the euglenas were injected.
The same observation can be made when the depth of the swimming pool is 0.1mm that is to say about twice the length of the euglenas. *Source: The experiment was done on 2 June 2014 at the University Pierre and Marie Curie (IMPMC). Many thanks to Dr. Céline Férard for her help.*

Can the formation of such a lattice be interpreted as a proof of the existence of inter-individual interactions? The best we can do to get a clearer insight is to discuss briefly other (better known) cases of lattice formation. We will start with the simplest case and then move by steps to the more complicated cases.

First we will discuss the static situation of an isolated system which occurs in the formation of crystals. Then we discuss the formation of snowflakes which is a static situation for a system that is not isolated. Finally we discuss bioconvection which is a dynamic effect in a system subject to an exogenous factor (light).

Crystals

In physics, more precisely in crystallography, observed lattice patterns are the result of inter-atomic interactions.

As an illustration, one can mention the case of solid sodium chloride, NaCl, which forms a simple cubic lattice in which sodium and chloride ions alternate with each other. Every positive sodium ion Na^+ is surrounded by 6 negative chloride ions Cl^- and vice versa. The surrounding ions are located at the vertices of a regular octahedron. This lattice is called a face-centered cubic lattice.
There is of course an electrostatic attraction between the positive and negative ions. However, the fact that a Na^+ ion is in equilibrium (except for small vibrations) should not be interpreted as resulting from the fact that the attractions of the 6 chloride ions cancel each other for this would lead to an unstable equilibrium. In fact, the equilibrium results from the existence, in addition to the electrostatic interaction, of a strong short-range repulsive force.
One may wonder why sodium chloride forms a face-centered lattice whereas cesium chloride forms a body-centered lattice. The reason is in relation with the size of the ions and the range of their ionic interaction. The space allowed for the sodium ion is determined by the ion radius of the sodium chloride and by the geometry of the lattice. Sodium has 11 electrons, whereas cesium has 55. Thus, to fit into the crystal, cesium needs a different geometrical arrangement than sodium.

In short, one can keep in mind that for an isolated system the formation of a lattice is due to inter-individual interactions and that the shape of the lattice reflects individual characteristics such as size and interaction range.

Snowflakes

For a system subject to exogenous factors the situation is more complicated. An obvious illustration is given by the shape of snowflakes. Although all snowflakes consist of crystals of ice, they occur in a great variety of shapes. Why?
Several exogenous factors such as temperature or the density of water dropplets will affect the formation of snowflakes. Moreover, between their formation and the moment when they become big enough to be released from the cloud, snowflakes experience a growth process which can last from a few minutes to several hours depending on conditions[23]. The final shape will reflect both the shape of the seed-crystal and the conditions experienced during the duration of growth. For instance, initial crystals with a thin edge grow faster, ultimately leading to large, thin flakes, whereas snowflakes that begin with blunt edges grow more slowly and eventualy lead to small, thick flakes.

Bioconvection

[23] In laboratory conditions for a tiny crystal of ice to grow into a snowflake may typically take about 45mn.

Ever since the first systematic observations done by Wager in 1911, the lattices formed by the euglenas were attributed to the phenomenon of bioconvection. The models of bioconvection rely on the so-called Rayleigh-Taylor instability. This hydrodynamic effect occurs when a liquid of density ρ_1 forms an upper layer atop of a liquid of lower density $\rho_2 < \rho_1$. For instance, in the experiment described in Plesset and Winet (1974) $rho_1 = 1.008$ (corresponding to a concentration of euglenas of $c_1 = 1,400$ per cubic-mm) and $\rho_2 = 1.007$ (corresponding to $c_2 = 560$ euglenas/cubic-mm).

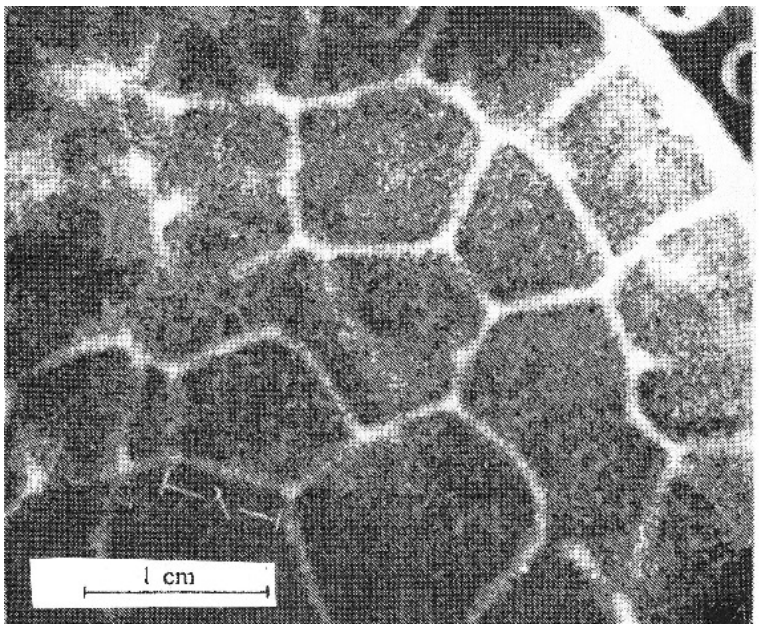

Fig. 18b Formation of a network of euglenas (seen from above). The "swimming pool" of the euglenas was 12mm deep and the upper layer had a thickness of 1.5mm (the role of the depth with respect to the upper-layer remains somewhat unclear). The authors do not give the level of light. It is said that the "photographs were strobe-illuminated" but we do not know what was the level of light between the observations. The authors also do not say how long the network remained unchanged. *Source: Adapted from Plesset and Winet (1974).*

Plesset and Winet say that the onset on instability is characterized by a pattern of falling fingers which is maintained by "return upward swimming" of the organisms. They explain the upward swimming by the fact that the micro-organisms under consideration (namely *Tetrahymena pyriformis*) have negative geotaxis that is to say a tendency to go upward. Yet, why should organisms which have such an upward swimming tendency let themselves fall to the bottom once they have reached the surface. In other words what we have here is the assumption of a dual behavior. When they fall to the bottom, the organisms behave like inert particles, yet when they swim upward they behave like living organisms. Does the transition from one state to the other not require an explanation?

The explanations set forth by Harold Wager (1911) to account for the patterns he observed with *Euglena viridis* raise the same difficulty. Just as *Euglena gracilis*,

Euglena viridis has positive phototaxis (i.e. it is attracted by light) up to a given intensity threshold above which it has negative phototaxis. Yet, this property played no role in most of the experiments done by Wager because they were performed in darkness. Light was applied only for taking pictures. Consequently, phototaxis cannot be invoked to account for the downward or upward movements of the euglenas.

On the contrary, in more recent expriments (e.g. Suematsu 2011) the phototactic property of the euglenas plays a role because a strong light is applied under the sample. Thus, the upward movement is easily explained through negative phototaxis whereas the downward movement may possibly be explained by another effect such as self-shading or the fact that (for some reason) the euglenas stop swimming

The experiment reported at the beginning of this section according to which a lattice pattern appears (i) in darkness and (ii) in a swimming pool of depth 0.1mm or 0.5mm suggests that one needs another explanation than bioconvection. A depth of 0.1mm means that there are at most 2 or 3 layers of euglenas. In other words such a case can probably be approximated as a 2-dimensional problem. As in addition no exogenous factors (sich as light) needs to be taken into account, the situation is much simpler than bioconvection. Moreover, as the lattice remains unchanged for several hours it is legitimate to assume a stationary state.

Two compartment experiment

A two-compartment experiment with ants was described earlier in the section about clustering. It is natural to try it also with euglenas. The figure below describes the device that was used[24].

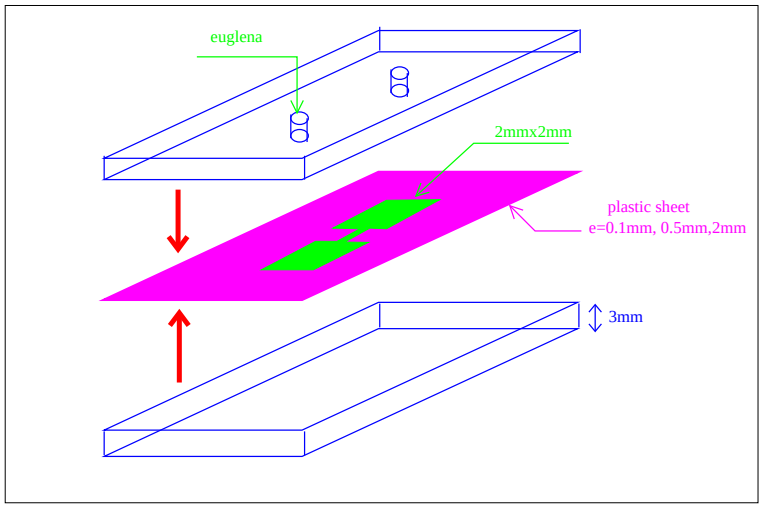

Fig. 19 Size and sandwich structure of the two-compartment swimming pool.

The main challenge was to fill the swimming pool with euglenas but without air

[24]In fact we used the same device for the lattice experiment because one experiment was in fact the continuation of the other. For lattice formation the fact of having two compartments played no role. It had just the advantage of giving two parallel observations instead of a single one.

bubbles. The technique depends on the depth. Thus, for a depth of $e = 0.5$ mm the droplets injected through one hole spread to the hole pool, whereas for $e = 0.1$ mm increased friction hinders the spreading. In this case the euglenas must be introduced before the sandwich is closed; a layer of grease will prevent any leakage in this first phase. Once the device was ready with the euglenas inside the two holes were closed to prevent any evaporation.

The experiment was tried for 3 depths, namely $e = 0.1, 0.5, 2$ mm. It led to the following observations.

- Immediately after the device was filled and ready it was put in complete darkness under a metal box. In order to make an observation the box was opened at time $t = 15$mn and $t = 30$mn. For $e = 0.1$mm and $e = 0.5$mm a lattice formed, as already described. For $e = 2$mm no lattice formed.
- Once formed the lattice remained identical for several hours.
- In the following hours (in most experiments this was during night-time) the lattice disappeared and was replaced by a smooth and uniform distribution. Although the distribution was fairly uniform on each side, the concentration was not the same. Most of the euglenas were located one side.
On the contrary, for $e = 2$mm the density remained the same on each side.

In the following weeks these experiments will be repeated with special attention given to the role of the initial concentration of the euglenas.

Acknowledgements This work is basically a collective undertaking. This is already clear from the fact that it has several co-authors, but there were many other persons who provided help and advice whose names do not appear in the co-author list. We would particularly wish to thank Prof. Avraham Be'er of Ben Gurion University and Prof. Hiraku Nishimori of Hiroshima University who shared with us their enthusiasm for this kind of research as well as Dr. Céline Férard of the IMPMC (University Pierre and Marie Curie) whose help was invaluable.

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[This is a long review paper which contains a few photographs and about one hundred references.]

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[25] *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"

isms cultures as an example of Rayleigh-Taylor instability. Nature 248, 441-443.

[This seems to be one of the earliest papers in the investigation of bioconvection based on the Rayleigh-Taylor instability. It can be noted that such a mechanism was already suggested by Wager (1911) although he did not explicitely mention Rayleigh-Taylor.

In the present experiment, the depth was 12mm and the length of the microorganisms was similar to the length of *Euglena gracilis* that is to say about 50 micrometer. Started in the 1970s, this stream of research extended well into the 1990s and 2000s.]

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[This is an early paper on bioconvection. As it is a long paper (58 p.) which contains many pictures and drawings one expects a fairly comprehensive description. For *Euglena viridis* which is of the same size as *Euglena gracilis* aggregation seems to occur only in darkness which is the opposite of what is observed in *Euglena gracilis*. This is perhaps related to the fact that the threshold for the shift from positive to negative phototaxis seems to be much higher.

A great number of experiments are described, but usually they do not have a well defined purpose and their description is rather loose. Again and again one reads that the experiment was done in a “shallow vessel” without any depth indication.

The same remark applies to the conclusion: “The regularity of the aggregation depends upon the depth of the cell and the number of organisms present. It is more regular in a shallow vessel than in a deep one”. That conclusion immediately raises the question of whether there is a depth for which the aggregation is maximum and whether or not there is a critical depth below which the aggregation disappears. Unfortunately, these are questions that the author did not address.]

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[This thesis is available on line; it is not about *Euglena gracilis* but about two similar microorganisms, namely *Chlamydomonas nivalis* and *Chlamydomonas reinhardtii*. In contrast with the euglenas which are cylindrical, those organisms are rather spherical. However, one would expect that the mathematical methods developed for this case can also be applied to the euglenas.]

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