Chapter 12
Apoptosis

In any society, whether human or non-human, there are links and interactions between individual units. A possible method for investigating these links is to consider what happens when they are severed. For instance, what will happen to an ant which is removed from its nest and kept in isolation in a place which offers the same conditions as the nest in terms of food, temperature, humidity, and other factors. Will it have a shorter or longer life than the ants which remain in the nest? If, as may be expected, its life is shortened, one would like to know *how much* it is shortened. Before answering this question (which we do later on in this chapter) it is important to realize its implications. This point can be illustrated through the following *gedanken* experiment. Suppose you are slicing bread and the knife slips. Your finger is cut and starts to bleed. You put a plaster and think nothing more of it. However, some skin cells have been displaced down into the muscle tissue; if they survive and divide they will produce skin cells in a location where no skin cells should exist. Fortunately, these displaced skin cells undergo self-destruction, a mechanism known as apoptosis[1]. Other expressions such as programmed cell death (PCD) or cell suicide (Raff 1998) are also used to designate the mechanism of self-destruction.

In order to establish a connection between our previous question about ants kept in isolation and the phenomenon of apoptosis one may wonder what happens when a skin cell is removed and kept in a test tube. Martin Raff, a pioneer in the study of apoptosis has performed numerous experiments of this kind. He was able to show

[1]The term “apoptosis” comes from a combination of two Greek words *apo* which means “from” and *ptosis* which means “falling”; in Greek the term apoptosis refers for instance to leaves falling from a tree.

that whereas cells can survive for weeks in cultures at high cell density, they undergo apoptosis at low cell density. This lead Raff to the conclusion that the only thing that stops living cells from ending their lives is the constant receipt of a biochemical signal saying “Stay alive, stay alive,...”. When living cells are put to grow in low density culture dishes they activate their death programme probably because the “stay alive” signals that they receive are too weak (Raff 1994, 1998).

In this chapter we first explain in what sense apoptosis is fundamentally different from necrosis which is another form of cell death. Then we examine why apoptosis is an important function for all organisms. A question of central importance is whether some form of apoptosis exists in populations which are not organisms. The very existence of organisms such as Dictyostelium discoideum (to which we come back later on) shows that the borderline between populations and organisms is not clear-cut but rather fuzzy and porous; therefore one would not be surprised to find apoptosis mechanisms also in populations. In a more general way one may conjecture that mechanisms similar to apoptosis may exist in all evolving networks for the purpose of eliminating links or nodes which are no longer serviceable.

1 Apoptosis versus necrosis

Cells can die in two different ways. (i) In necrosis the cell swells without any rearrangement of its internal components; eventually, the membrane of the cell is ruptured; the nucleus, DNA, mitochondria and all other internal building blocks are released and destroyed. Locally, the process results in an inflammatory reaction. (ii) In apoptosis, the cell first shrinks, its internal components are “packaged” in an orderly way before being released, engulfed and “swallowed” by neighboring cells. In short, apoptosis is an elaborate programme of self-destruction which results in orderly dismantling and reuse of cell components. Necrosis and apoptosis can be distinguished without ambiguity through microscopic observation (Fig. 12.1). How long does it take for the process of apoptosis described in Fig. 12.1 to be completed?

The answer depends on the type of cell but an order of magnitude is 10 to 20 hours.

Recognition of programmed cellular death as a development mechanism dates back well over 100 years (Clark et al. 1996). However, it is only in the last decades of the twentieth century that significant progress has been made in its understanding. Much of our current knowledge comes from work on Caenorhabditis elegans, a small worm about one millimeter in length which has 959 cells. In the course of its development 1090 cells are produced of which 131 die by apoptosis. The 2002 Nobel prize in Physiology and Medicine was awarded to Sydney Bremmer, H. Robert Horwitz and John Sulston for their discovery concerning genetic regulations of programmed cell death.

2 Role of apoptosis in the development of multicellular organisms

Apoptosis fulfills a number of important functions, but before examining them more closely it may be of interest to present an instance in which apoptosis seems almost purposeless. We already mentioned that because of its simplicity, C. elegans provides an ideal testing ground for the mechanisms leading to apoptosis. It was found that two specific genes control whether or nor a cell should survive. When these genes were inactivated the 131 cells which normally undergo apoptosis were able to survive in an adult worm. It turns out that the fact of having 1090 cells did not affect its survival capability. Thus, in this specific case, we see that apoptosis has no clearly defined function. However, there are many other cases in which apoptosis fulfills essential functions. One can mention the following.

- The resorption of the tadpole tail at the time of its metamorphosis into a frog occurs by apoptosis. Similarly, the formation of the fingers and toes of the fetus requires the removal by apoptosis of the tissue between them.
- One of the method by which lymphocytes eliminate virus-infected cells is by inducing apoptosis.

- Cells which for some reason are moved away from their normal location disappear by apoptosis.
- Cells which are damaged beyond possibility of being repaired and restored are eliminated by apoptosis.

It has been estimated that in a human organism from 50 to 70 billion cells die each day by apoptosis. It is interesting to compute the corresponding annual death rate. A human body has approximately 100,000 billion cells which leads to a rate of $365 \times 60\ 10^9/(10^{14}/10^5) =$ 21,900 per 100,000. This rate is about 1,000 times larger than the average annual suicide rate in human societies. However, we will see that suicide is only one of several mechanisms by which the functions normally performed by apoptosis can be achieved in human societies.

3 Apoptosis in plants

For fruit trees there is a kind of apoptosis mechanism through which fruits which are damaged are eliminated at an early maturation stage. As an example let us consider the premature drop of citrus. At first sight one may attribute them to weather conditions for instance rainfall or strong winds. However, a closer examination shows that weather is not the real cause. A first indication is the fact that premature fall does not necessarily affect fruits growing on high branches which are the most exposed to wind gusts. Moreover, the fallen fruit reveal a split at the navel end (that is to say the end of the citrus opposite to the stem end). Upon cutting the citrus one sees that there is a black discoloration due to a fungal organism (Grafton-Cardwell 2005) which suggests that it is the invasion of the fruit by a pathogen which provoked the separation of the fruit from the tree in the same way as as virus-infected cells are eliminated by lymphocytes. Other cases are reported in the literature which confirm the idea that the fruits which fall from trees are not selected randomly. For instance, it has been observed that prematurely fallen apples on average contain a smaller number of seeds than the apples which remain on the tree (Ulrich 1944).

4 Apoptosis in populations

Does apoptosis also occur in populations of social insects, in herds of mammals (e.g. herds of antelopes) or in human societies? The question is more difficult to answer than for cells because one can no longer rely on the clear-cut observational distinction between necrosis and apoptosis. There are two ways to get around this difficulty. The first is to observe entities such as social amoeba which are intermediate between populations and organisms. The second is to use Martin Raff's isolation technique.

- Amoebae are small living organisms consisting of only one cell. In ordinary conditions they behave as individual and fairly independent entities. However, under specific circumstances, for instance when subject to food deprivation, they may aggregate and form organisms of which they constitute the cells. In the case of the amoeba Dictyostelium discoideum one observes the formation of a slug-like organism in which the amoebae undergo a process of differentiation into spore and stalk cells. The interesting point from our perspective is the fact that the stalk is composed of dead cells that have undergone programmed death much in the same way as in cellular apoptosis. The fact that apoptosis does not seem to occur in populations of more or less independent amoebae but appears as soon as a global entity is formed clearly shows that apoptosis capability is a property of global organisms. In a sense it can be seen as the signature of a high level of interdependence between the units composing the organism. In the next paragraph we test this conjecture in the case of populations of social insects.

- It may be remembered that in Raff's experiments, when cells were grown in low density cultures the cells activated their apoptosis program and died. In May-July 1944, Pierre-P. Grassé and Rémy Chauvin performed similar experiments with insects. Their outcomes are summarized in Fig. 12.2. To our best knowledge, this kind of experiments has not been repeated which is fairly surprising given the great interest in these questions in recent decades.

Grassé and Chauvin describe their experiments as follows. Bees from the same bee hive were captured on leaving the hive and were put into small boxes (150 cm^3 in volume) fit with a wire mesh lid. Different boxes were prepared with a single bee or with 2, 3, 5 and 10 bees. All of them received plenty of food in the form of water sweetened with saccharose. The authors say that they used a total of 250 bees but they do not specify how many boxes of each sort were prepared; therefore we do not know the margin of error of the experimental points. A similar experiment was performed with ants. In this case a total of 105 worker ants from three different nets of the species *Leptothorax* were used. The boxes consisted of small cavities in a block of plaster whose humidity was maintained. The ants were nourished with wood powder, saccharose and tiny bits of grasshoppers. A second experiment was performed with ants of the species *Formica rufa*. A total of 120 ants were captured from the same nest and kept in groups of 1, 2, 3, 5 and 10. Every two days they received fragments of a ripe apple. Similar experiments were performed with wasps (*Polistes gallicus*) and with termites (*Reticulitermes lucifugus*).

The life expectancy of the insects in the isolated subgroups is described in Fig. 12.2. Two observations can be made.

1) In all cases the life expectancy of a single individual does not exceed 20 days.

2) Except for the wasps, the life expectancy is greater for the group of 10 than for the groups of 1 and 2 individuals. This suggests that as in Raff's experiments, the life expectancy is density dependent. As a conjecture we suggest that it is in fact a function of the strength of the interaction. When the insects are in their nest the interaction is maximum, it subsists in weak form in groups of 10 and it is minimum for single individuals.

If the previous conjecture is correct, this kind of experiment would give us a method for measuring the strength of the interactions in a population. Thanks to the studies of entomologists and ethologists in the past 50 years we gained a good understanding of the means of communication of social insects. Yet, we still know very little about the strength of the interactions. There are certainly marked differences in interaction strength between different species of ants but we are unable to estimate them. For instance, we know that the exchange of food between individuals, an interaction that entomologists call trophallaxis, is an important way of communication. We also know that trophallaxis is more important for termites and ants than for bees. Yet, the results in Fig. 12.2 show that of the five species, bees are the most vulnerable to isolation. This would suggest that apart from trophallaxis bees have other ways of communication which are as important[2]. Another intriguing question concern the results obtained for the wasps. Grassé and Chauvin say that they repeated this experiment four times and that the results consistently showed that a larger subset is *not* conducive to a longer life expectancy. How can one understand this exception? Grassé and Chauvin left the question open until additional experiments provide some clues. For instance, it would be of interest to know if these results hold for other species of wasps or if they are specific to *Polistes gallicus*.

Although the pioneering work of Grassé and Chauvin is cited in some recent research papers, it is not seen as opening an avenue of research that should be pursued. This attitude is probably due to the fact that there has been a notable shift in the interest of researchers. Nowadays, the emphasis is on detailed descriptions of interaction mechanisms[3] rather than on the kind of comparative studies initiated by Grassé and Chauvin. From a system science perspective this methodological choice appears perplexing. Are detailed descriptions really conducive to a better understanding? History provides myriads of detailed descriptions from which little overall understanding can be gained unless the information is analyzed from a comparative perspective. In sociology, the fact that we are able to know social interactions in all

[2] We know that bees have elaborate communication techniques for sharing information about floral patch feeding sites, but it is not clear if these ways of communication are also used for other purposes.

[3] The papers by Boulay et al. (1998, 1999, 2000) are representative of the this approach.

their minute details proves more an embarrassment than an asset. In all these fields choosing the “right” level of description is a crucial step. Physicists and chemists restricted themselves to a fairly schematic view of the complex mechanisms of interaction at the molecular and atomic level; in contrast, all their efforts were devoted to developing a comparative analysis of physical phenomena which highlighted the central role of a few simple mechanisms. As one knows, this approach proved highly successful.

5 Apoptosis in groups of mammals

What is the effect of social isolation on life expectancy in groups of mammals. As a preliminary observation it can be noted that many mammals such as gazelles or antelopes live in fairly small groups of 10 to 15 individuals and form large herds only in specific circumstances for instance during their migrations. The same observation applies to birds. The large groups of birds that can be seen in nesting areas are gatherings of individuals rather than societies of highly interconnected entities. The colonies of prairie dogs in the United States or Mexico are one of the few groups of mammals which compare with colonies of social insects. Yet, even large “towns” of prairie dogs contain less than 5,000 individuals whereas nests of ants or termites may contain several million individuals. Moreover, they are an assemblage of interconnected family units rather than wholly integrated societies.

There are reports of high death rates for dolphins kept in marinas especially during the first months of captivity. However, it is difficult to know whether these deaths are due to social isolation or rather to the small size of the pools. One should remember in that respect that dolphins swim up to 80 kilometers a day and dive at depths of 200 meters[4].

[4] As a matter of comparison, dolphins can legally be kept in tanks with an area of 8 meters $\times$ 8 meters and a depth of only 2 meters.

6 Apoptosis in human societies

The main message of the previous sections is that the more integrated an organism the more important it is the eliminate “anomalies”, a word which should be taken in a broad sense which includes for instance (i) a cell which has been displaced to a wrong place (ii) a cell which has been damaged beyond repair (iii) a cell which has been penetrated by a virus or an ant which has been infected by a parasite. We underlined Martin Raff’s interpretation in which “stay alive” signals are received permanently by any cell. The experiments performed by Grassé and Chauvin suggest that there are similar mechanisms in populations of social insects. In this last section we wish to discuss briefly the possible implications for human societies.

It is not unreasonable to suppose that the existence of highly integrated organizations such as churches, armies or firms would be imperiled by the presence and growth of “anomalies”, but what precise meaning should we give to this word? In the context of a church, a person who does not share the common creed certainly represents an “anomaly” which should be removed. The removal can occur in different ways.

- Excommunication is the most common way. For instance, in Amish[5] communities the concept of church discipline plays a key role. When unrepentant dissidents are excommunicated they are shunned and rejected by their community even in ordinary life. Some Amish have even be calling for the suspension of the marital tie when one of the partners has been excommunicated; in their view, church discipline should have precedence over the sacrament of marriage. Confronted to such a broad ostracism dissidents have no other choice than to leave the community. In this case apoptosis takes the form of exclusion (Nolt 1992).

- Back in the 16th, 17th and 18th centuries, when religion played a key role in Western societies, the removal of dissidents often lead to their execution. Apart from the case of the Papal inquisition which is well known one can also mention the

[5] The Amish are Anabaptists, a radical wing of the Protestant Reformation movement; their main implantation is in Pennsylvania.

Penal Laws in Britain. Enacted between 1559 and 1580 they provided the penalty of high treason for the third offense which means that unrepentant dissidents were dispossessed of all lands and goods before being executed. Moreover, from 1563 to 1736 witchcraft was an offence punishable by death in Britain; as an example of this kind of episodes one can mention the witch hunt that took place in East Anglia between 1643 and 1647 and resulted in the execution of about one hundred witches (Gaskill 2005). For the whole of Europe the total number of witch trials which are known to have ended in executions is around 12,000 (Wikipedia, article "Witch trial").

- As a striking illustration of the religious climate in the middle of the sixteenth century, one can mention that a choral in a cantata by J.S. Bach (BWV 126: *Erhalt uns, Gott, in deinem Wort*, i.e. "Lord, keep us in your word") contains the following prayer (my translation): "Lord, keep us in your word and direct death at the Pope and Turks who try to tear from his throne Christ Jesus your son" [6] .

- Military discipline is fairly harsh because "dissidents" must be removed before they are able to contaminate the rest of the unit. The fact that in time of war the death penalty has been commonly used should not come as a surprise. Clearly, in such a situation exclusion or imprisonment would not be an effective deterrent.

- In contrast, firms and corporations can rid themselves of "dissidents" simply by discharging them.

- The issue is much more difficult for schools. One of the main purposes of schools is to give a degree of cultural homogeneity. Of course, unrepentant "dissidents" can be expelled from their schools but they cannot be completely excluded from the education system at least not until they are old enough to work. This implies that the education system must develop institutions that are able to handle "dissidents". The less homogeneous the audience, the more frequent the manifestations

[6] The text, which is due to Martin Luther, reads in German as follows: *Erhalt uns, Herr, bei deinem Word, und steuer des Papsts und Türken Mord, die Jesum Christum, deinern Sohn, stürzen wollen von seinem Thron.* Bach's cantata was played for the first time on February 4, 1725.

of dissidence and the more important the institutions which are in charge of "dissidents" [7].

In short, for institutions the phenomenon of dropout, whether voluntary or forceful, plays the same role as apoptosis in multicellular organisms. Can one apply the same kind of argument to the society? An illustration based on a concrete historical episode may give us an insight. Consider communities of Christians in Japan around 1600. Historical accounts show that Christians were not well accepted by the rest of the population. In such a situation there were several possible outcomes depending on the specific situation.

1) In the face of growing hostility, Dutch or Portuguese traders or settlers may decide to leave Japan and return to Europe.

2) Japanese Christians may be tempted to move to isolated place and form closed communities which will have minimal contact with non-Christian Japanese.

3) In 17th century Japan the religion of the people was to a large extent determined by the religion embraced by their lord. As a result, the defeat and overthrow of a Christian warlord had the likely effect that his subjects would return to Shintoism.

4) In contrast to what happened in Europe for Puritans, Quakers, Anabaptists and many other religious minorities, emigration does not seem to have been a real option for Christian Japanese. Thus, if none of the previous solution was adopted, there remained the possibility of committing suicide.

5) Finally, persecutions and pogroms may occur to the effect of eliminating the Christian dissidents. History tells us that there were several episodes of this kind in the early 17th century, for instance in 1614, 1626, 1637 (Quid 1997, p. 1277).

The previous discussion shows that suicide is only one of the possible responses to a situation of social isolation. Earlier in this chapter we noted that in a human organism

[7] As an illustration one can mention the fact that for 15-17 year old youths the dropout rate is 16% for recently arrived immigrants and 5% for immigrants who arrived in the U.S. during early childhood (Fry 2005).

the apoptosis rate is about 20,000 per 100,000. A social parallel of this rate would include all mechanisms through which “anomalies” can be eliminated; for instance in the case of 17th century Japan one would have to sum up the rates corresponding to the five previous mechanisms.

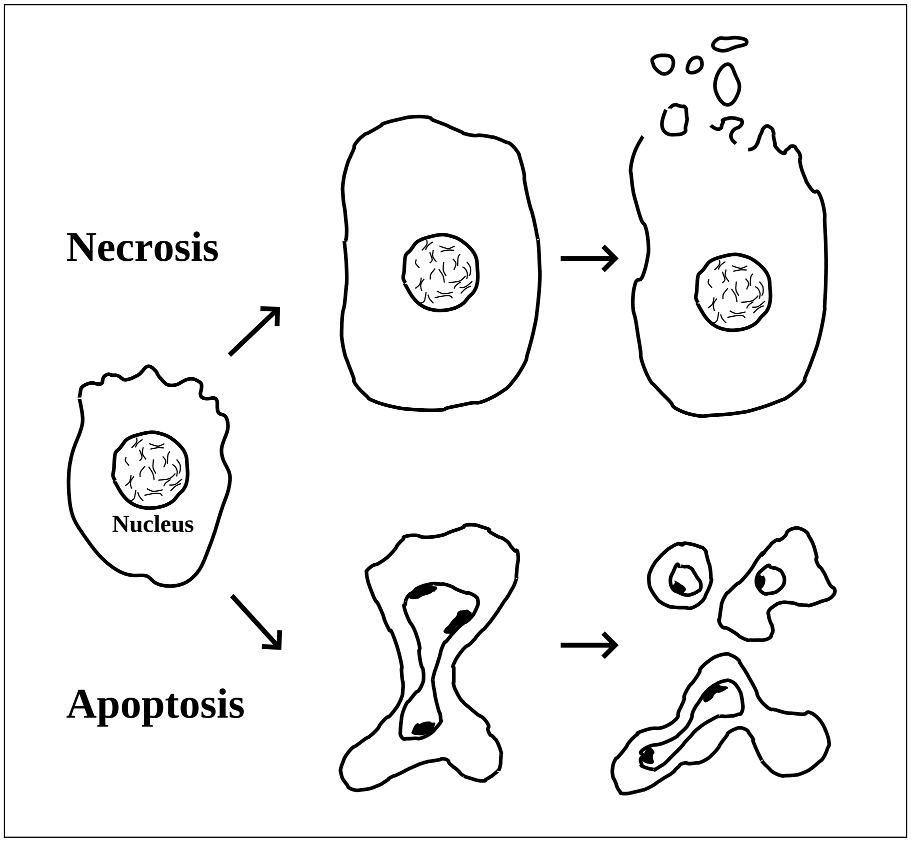

Fig. 12.1 Necrosis versus apoptosis. Necrosis is an explosive cell death that results from severe injury and is characterized by swelling, cell rupture and release of the cell components in a way which can damage nearby healthy cells. Note that the DNA remains dispersed throughout the nucleus. Apoptosis (also called cell suicide) is a controlled death process in which the cell components are “packaged” and ingested by nearby cells.

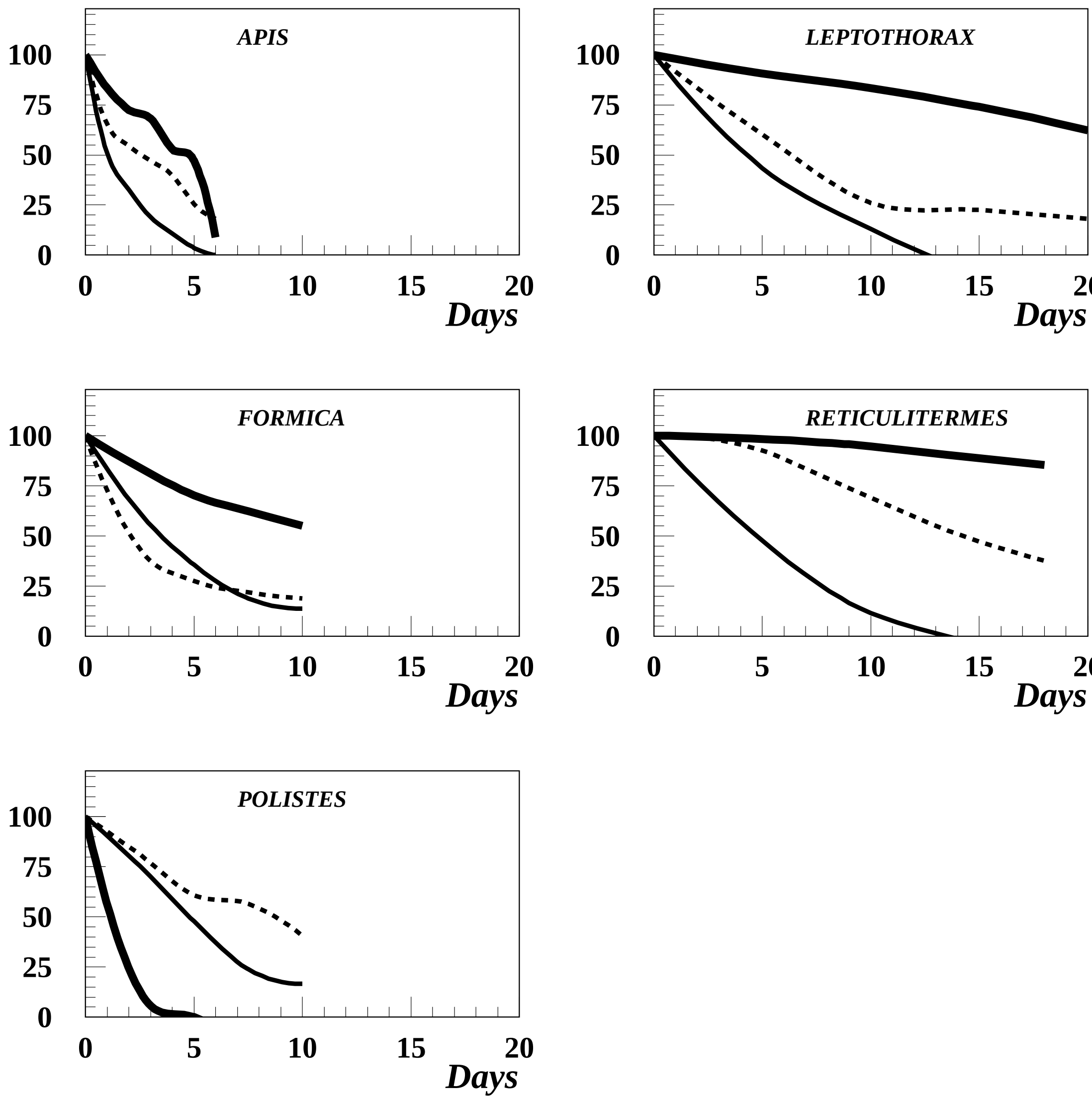

Fig. 12.2 Effect of removing insects from their colony. Several groups of 1, 2 and 10 insects were removed from their nest and kept in isolation. They received plenty of food and were kept in conditions which matched as closely as possible those in the nest. The vertical scale shows the percentage of surviving insects in each group. The thick solid lines represent the single insects, the broken lines represent the groups of two insects and the thick solid line represents the groups of 10 insects. Initiated in 1944 by P. Grassé and R. Chauvin this kind of experiment may provide a methodology for gauging the strength of the interactions in the colony. *Apis* designates a species of bees, *Leptothorax* and *Formica* are two species of ants, *Reticulitermes* are termites and *Polistes* are wasps. The insects in isolation received plenty of food and were kept in conditions which matched as closely as possible those in the nest. *Source: Grassé and Chauvin (1944).*