Our postman said ... "Isle of Wight disease? Never heard of it. My bees? No, I never lost none. John Preachy's? Why, of course they died; he used to feed 'em on syrup and faked-up stuff all winter ... You can't do just as you like with bees. They be wonderful chancy things; you can't ever get to the bottom of they."

Adrian Bell (*The Cherry Tree*)

HONEY BEE PATHOLOGY

Second Edition

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PREFACE

This book incorporates much that has been learned in recent years, including knowledge of diseases and pathogens that were previously unknown, or were believed to be localized but have proved to be widespread and common. The discovery of some of these has caused much concern; new anxieties have arisen world-wide, and controversies of long ago in Europe have recently been rekindled in North America.

Most books about bees discuss them with little or no regard for other insects. This is an artificial separation which, although reasonably based on human interests, has often led to unreasonable anthropomorphic attitudes about bees, especially about their diseases. It can be corrected to some extent by considering honey bee pathology in the context of insect pathology. This subject has become too extensive and diverse to be summarized readily, and it is only touched upon in this book; but an awareness of it can give perspective and scale to a detailed account of the pathology of bees. This may well modify in return, some of the attitudes that prevail about insect pathology, many of which have often been influenced by well-established but erroneous beliefs about the diseases of bees.

Although much has developed in honey bee pathology since 1981 the treatment in this book is selective for the sake of brevity. Whenever possible, references are given to review and comprehensive papers where details can be found on special points.

Some knowledge of biology on the part of the reader is assumed, but, for those who are unfamiliar with biological terms, inexpensive scientific and biological dictionaries should be adequate.

Advanced accounts of the anatomy of bees are given by Snodgrass (1956) and Dade (1962). Wigglesworth (1972) and Roeder (1953) include much information about bees in their works on insect physiology.

We are indebted to many friends and colleagues, both scientists and beekeepers, at home and abroad, for their help and stimulating discussions. In particular, we thank Lynda Castle and Dr. J. Philip Spradbery for many illustrations.

Leslie Bailey
Brenda V. Ball

1
INTRODUCTION

Man has concerned himself about the diseases of honey bees for thousands of years. Aristotle (384–322 B.C.) described certain disorders, and Virgil and Pliny referred to some about the beginning of the first millennium. None of their descriptions is sufficient to identify the disorders with certainty. However, they made it plain that bees then were much the same as now and that the diseases we today call foulbrood and dysentery probably existed in antiquity. One description by Aristotle of a disorder of adult bees corresponds with that of one of the syndromes of paralysis (Chapter 3, I.).

In the more recent past, Shirach in 1771 described "Faux Couvain" (Steinhaus, 1956), which may well have been American or European foulbrood; and Kirby and Spence (1826) described "dysentery". Soon afterwards occurred one of the most significant events in insect pathology, and one that greatly influenced the concept of infectious diseases of all kinds, including those of bees. This was the demonstration by Louis Pasteur, in the mid-nineteenth century, of the way to rid the silkworm, *Bombyx mori*, of "pebrine", a disease that was crippling the prosperous silk industry of France. He and his colleagues recognized the pathogen, which was later named *Nosema bombycis*, observed that it was transmitted in the eggs from infected females and, by microscopically examining the progeny of quarantined females for spores of the pathogen, were able to select healthy stocks and re-establish productive silkworm nurseries. Pasteur was greatly honoured by the silk industry and the French government for his classic solution of their problem. He, and others strongly influenced by him, went on from this success to establish the basic principles of infectious diseases of man and his domesticated animals. All kinds of severe diseases soon were found to be due to micro-organisms or viruses and the hunt for these became the dominant feature of disease investigations.

Great hopes and expectations then arose about the diagnosis and cure of bee diseases. Dzierzon (1882) recognized that there were two kinds of foulbrood of bees: "mild and curable" of unsealed brood (probably European foulbrood), and "malignant and incurable" of sealed brood (almost certainly American foulbrood). Microbiological investigations into them were begun by

Cheshire and Cheyne (1885). Entomologists also became impressed by the idea of spreading pathogenic micro-organisms among pest insects, hoping to control them with diseases as destructive as that which had ravaged the French silk industry and as those believed to be rife among bees.

The parasites that were newly found in sick bees quickly led to a common belief that bees suffered from a wide range of infections of great severity and that the presence or absence of serious infectious disease was simply a matter of the presence or absence of a pathogen. When a pathogen was present severe disease and eventual disaster were thought to be certain, as had first been shown with pebrine in the silkworm and with several diseases of other domesticated animals and of man. In fact, although many of the pathogens of bees usually kill the individual they infect, or at least shorten and otherwise disrupt its life to some degree, their effects on colonies are generally less predictable, which gives rise to dilemma and controversy about their importance and how best to deal with them. Nevertheless, precautionary measures and treatments have always been sought, often desperately; and there has been a degree of success, although this has often been achieved by little more than chance and leaves much to be desired.

Honey bee pathogens comprise a wide variety of types, each being a special case with its own range of characteristics. The best methods of control will take account of these traits. Accordingly, the likelihood of devising such methods can only be increased by more knowledge of the nature of each pathogen and of its environment—the honey bee colony.

2
THE HONEY BEE

I. NATURAL HISTORY

The honey bee colony has frequently been regarded either as an ideal society or as a kind of totalitarian state. It is neither. Social insects, whether termites (Isoptera), wasps, ants or bees (Hymenoptera), do not form organizations analogous to those of human societies. Their colonies are no more than families, often very large ones, but usually comprising one long-lived fertile female and her progeny; and each family is an independent unit which needs no contact with others apart from the occasional pairing of sexual individuals. Regarded in this way, social insects are not very different from the several million other known species of insects with which they form an intrinsically uniform group, especially with regard to their fundamental structure, physiology and pathology.

However, notwithstanding their close relationship with other insects, including some 10 000 species of bees of which about 500 are social, two of the four major species of the genus *Apis*, the true honey bees, are sufficiently distinct to have long attracted the special attention of man. These are the European honey bee, *Apis mellifera*, and the very similar but physically smaller and quite distinct species, the eastern honey bee, *Apis cerana*. These two honey bee species have long been of particular interest to man because they store large amounts of accessible honey and can be induced to nest in movable containers or "hives". During the past few hundred years, the European honey bee has been taken by man all over the world and with particular success to the Americas, Australia and New Zealand. There are also several strains of *Apis mellifera* naturally distributed throughout the African continent. The eastern hive bee is restricted to S.E. Asia, China, east USSR and Japan, and is to some extent being replaced by *Apis mellifera*, particularly in the temperate zones of these regions, by the activity of beekeepers.

A colony of honey bees is headed by a single queen and is composed of about 50 000 individuals on average. Worker bees clean and make the wax

combs and feed brood in their first week or so of life, and then begin to forage, usually when they are 2 or more weeks old, first for pollen and then for nectar. They live no more than 4 or 5 weeks in summer, but in autumn, when nectar-flows and brood-rearing end, they hibernate as a cluster and individuals of the cluster may survive as long as 7 months. There are usually a few hundred drones in colonies in summer whose sole function is to mate with virgin queens. Drones mate only in flight, frequently with queens from colonies several miles distant from their own. They are ejected from the colony by worker bees in autumn before the winter cluster forms.

Colonies reproduce by swarming. This usually means that the queen leaves the colony in early summer, attended by many, possibly more than half, of the workers, and goes to another suitable nest-site. The queenless colony that remains rears further queens, the larvae of which are usually being prepared at the time the swarm leaves. The first of these new queens to emerge usually kills the others before they emerge and thus becomes the new reigning queen. Within a few days she mates with a number of drones and stores sufficient spermatozoa in her spermatheca for her lifetime of 3 or 4 years. These spermatozoa are either released, a few at a time, to fertilize each mature egg just before it is laid and produce females (workers and queens), or they are withheld and the resulting haploid eggs become males (drones).

When by any chance a colony loses its queen, a new one is reared from a young larva which would otherwise have become a worker; but it is not known how a worker larva changes its development to become a queen.

The larval worker bee passes through the following six distinct phases in its life (Fig. 1):

1. The embryo develops for 3 days in the egg, which is fixed to the base of an open cell in the comb.
2. When the larva hatches from the egg it is fed continuously for the next 5 days, while it is growing in the open cell, by young adult bees or "nurse bees". The larva sheds its skin about every 24 h. The mid-gut of a growing larva is a blind sac (Fig. 2).
3. The fully grown larva is sealed in its cell by nurse bees and then spins a cocoon. This is discharged as a fluid from an orifice on its labium-hypopharynx or "lower-lip", and smeared over the cell walls where it becomes dry, tough and papery. At the same time the larvae discharges its faeces via the rectum, which temporarily joins up with the mid-gut for this purpose. The faeces become sandwiched between layers of the cocoon. About 2 days after it is sealed over, the larva lies on its back with its head towards the cell capping.
4. The quiescent larva changes within a loosened fifth skin to a propupa,

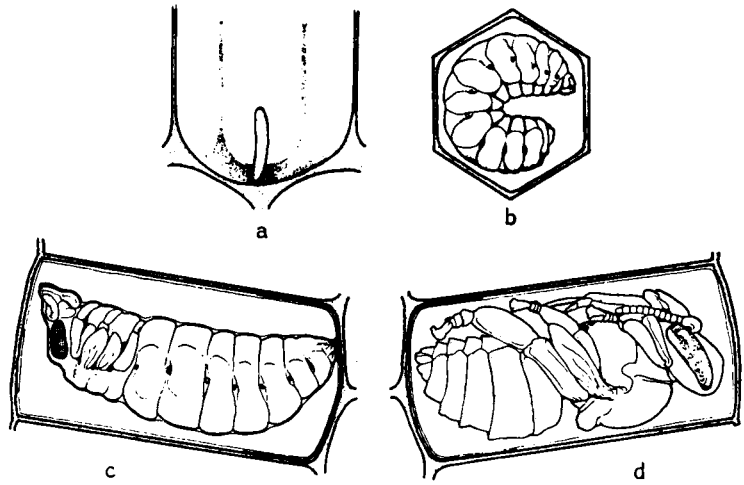

Figure 1 The stages of development of a honey bee: (a) egg on the base of a cell in the comb; (b) larva about 4 days old in its open cell; (c) propupa and (d) pupa in their capped cells.

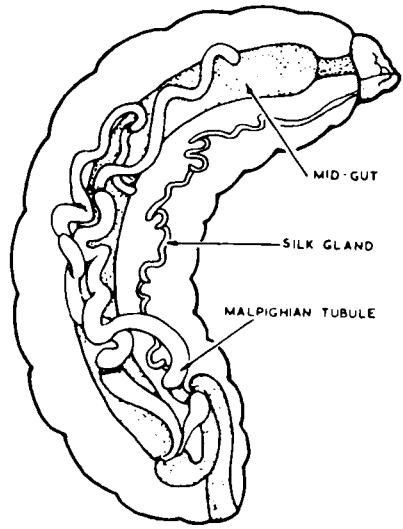

Figure 2 Anatomy of the young larval honey bee. The mid-gut, hind-gut and Malpighian tubules are blind at their junction at this stage. (After Nelson, 1924.)

and after 2 days of this phase it sheds the fifth skin to become a white pupa.

5. The pupa, now resembling an adult bee in shape, slowly darkens in colour, beginning with the eyes.
6. The pupa sheds its skin, and a few hours later the adult insect emerges from its cell.

The pupal stage is shortest for the reproductive caste, "queen", and longest for the male, "drone". Queens emerge from their cells about 16 days after the egg is laid; the worker bees, which are genetically similar to queens but have undeveloped ovaries as well as other morphological differences, take about 21 days; and drones take about 24 days to develop. Drone larvae stay unsealed for about 2 days longer than worker larvae.

The adult bee eats pollen and honey, the latter being floral nectar concentrated by evaporation and with its sucrose content inverted by enzymes from the hypopharyngeal glands of adult bees until it is virtually an aqueous solution of about 30% glucose, 40% fructose, 8% maltose and other disaccharides, 2% sucrose and 0.5% organic acids. Pollen supplies all the protein fraction of the food and is eaten mainly by newly emerged and young adult bees in summer. The pollen is ingested into the crop in suspension in honey, from which it is separated, together with other particles, including those as small as bacteria, and passed into the mid-gut by the proventriculus. It is digested and absorbed by the gut and much of it is converted to a secretion of the hypopharyngeal glands of the head, from which it is discharged via the mouth as nitrogenous food for larvae, the adult queen and possibly for adult drones. Drones and queens are also able to feed themselves on honey, and drones probably feed themselves entirely in this way after their first few days or so of life. In autumn, when brood-rearing is almost over, protein is stored in the fat-body of adult bees as well as in the hypopharyngeal glands (Fig. 3). This reserve of protein probably helps the now rather inactive adult bees to survive the prolonged winter of temperate and sub-arctic climates and to have ready supplies of hypopharyngeal gland secretion for early spring brood-rearing.

Larval food may be a mixture of secretions from several different glands of the adult bee, but there is little doubt that most of the protein, which comprises 40–60% of the dry matter of larval food, is from the hypopharyngeal glands. Carbohydrate, which forms 30–50% of the dry matter of larval food, is probably entirely from honey: it may form a larger proportion of the food of older larvae but although generally believed, this remains to be proved. Pollen accumulates in the gut of the larvae, but the amount is insignificant compared with the nitrogenous needs of the growing insect and its presence is probably fortuitous. Larval food like honey, is acid, the usual pH being

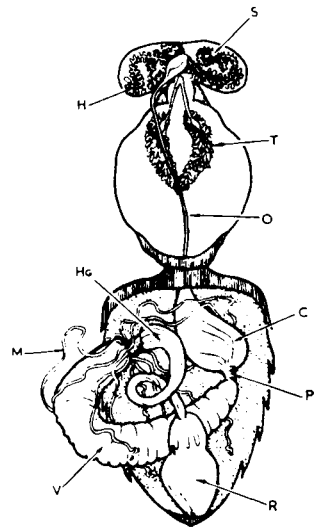

Figure 3 Glands and viscera of the adult bee:
C = crop, H = hypopharyngeal glands, Hg = hind-gut, M = Malpighian tubules, O = oesophagus, P = proventriculus, R = rectum, S = head labial glands, T = thoracic labial glands, V = ventriculus (mid-gut). (After Snodgrass, 1956.)

about 4.0; 5–20% of the dry weight of larval food is fatty material. Much of this is 10-hydroxydecenoic acid which is bactericidal at the normal pH of the food and comes from the mandibular glands.

II. BEEKEEPING

The honey bee evolved to the state in which we know it today long before the advent of mammals, not to mention man. Yet it is a popular belief among many biologists as well as beekeepers that bees are domesticated. The only insect that has been domesticated is the silkworm, *Bombyx mori*, which needs the care and attention of man in order to survive. By contrast, honey bees are feral insects no less than any of the millions of other insect species living in the forests, countryside and gardens. Honey bees can and do survive independently of man. Indeed, they must be left at liberty, even when in the hives of beekeepers, in order to survive. We have not learned how to keep them isolated, even partially, from their environment, whereas many species of wild animals, including a great variety of insects, can be readily propagated

in entirely artificial conditions. Even if bees could be kept under such conditions, it would be of only academic interest because they would still have to be allowed to rove freely in order to collect nectar and to pollinate plants that need them. Beekeeping today is still as it has always been: the exploitation of colonies of a wild insect; the best beekeeping is the ability to exploit them and at the same time to interfere as little as possible with their natural propensities. The most productive strains of honey bee presently available for man are those that would survive best independently of him, because they are the ones that find and store most food. As will be seen, these basic requirements for successful beekeeping are also those for the best resistance of bees to their diseases.

Beyond providing a colony of bees with a weather-proof cavity of adequate volume in regions of abundant and varied nectar-yielding plants, the modern beekeeper can do relatively little that is beneficial for his bees, although he can readily do a great deal that is harmful to them. All the methods and paraphernalia of beekeeping are entirely for his convenience. Bee colonies can live successfully and indefinitely in a suitably sized cavity of no particular shape as well as in any beehive. Bees will successfully occupy hollow logs, drain pipes, baskets and more unlikely containers, as has been well known to beekeepers for millenia. All the refinements have come from the wish to

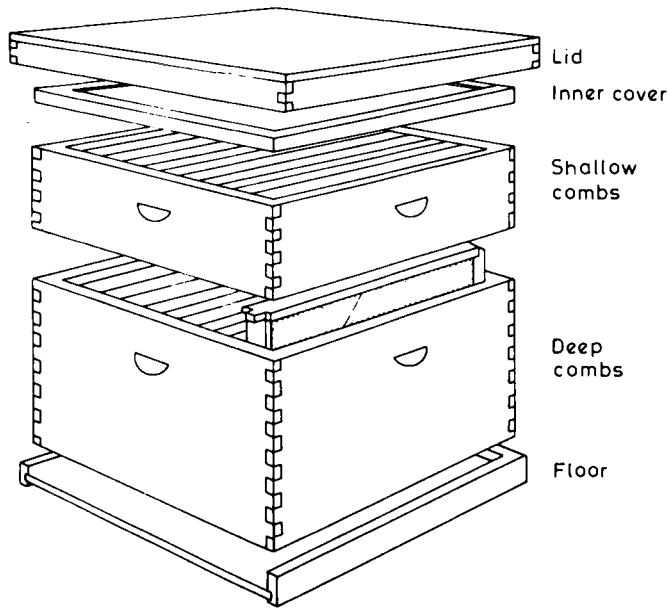

Figure 4 A modern beehive.

remove honey easily, with least harm to the bees, from colonies kept in readily transportable hives.

The ultimate achievement has been to make rectangular frames, usually of wood, in each of which bees will readily build one of their naturally orderly vertical combs (Fig. 34a). These frames are hung in a box, with a space of about 7–9 mm between the combs, and between the ends and top of the frames and the sides and top of the box. The bees accept this space as that of a thoroughfare and so do not usually block it up with wax and propolis, the way they quickly block narrower or wider gaps. The beekeeper can then easily remove, replace or rearrange the frames without much harm to the bees, and can extract the honey from the comb, usually in a special kind of centrifuge. This causes little harm to the combs, which are the items most valuable to the beekeeper and which can be returned to the hive for the bees to use again and again. Every significant feature of the several different kinds of successful modern beehive, however simple or complicated their wooden structure may be, is based on the existence of the bee space, which was first recognized by Langstroth in America in 1851.

Modern beehives (Fig. 4) are rectangular boxes of combs that have a loose lid and stand on loose floors. Each floor is constructed to form a narrow horizontal gap below the edge of the bottom box to form the entrance. Whole hives of this construction can easily be strapped up and stacked for transportation, and boxes of comb are simply piled one on another to make room as required for growing colonies and stored honey.

3
VIRUSES

Viruses are little more than genetic material enclosed in a protein shell or coat. They do not possess the mechanisms that would enable them to multiply independently by assimilating nutrients in the manner of most micro-organisms, such as bacteria; they can multiply only within the living cells of their host. When a virus infects a cell, it uses the cellular apparatus to make copies of itself. This can continue, without much obvious change to the cell, as long as the organism of which the cell is a part remains alive; but usually, infected cells become damaged, die and disintegrate, thereby releasing very many infective virus particles. These particles, or virions, are minute and usually far too small to be seen by light microscopy.

All forms of life are attacked by viruses, and insects of all kinds become infected by a wide variety of virus types. These are usually host-specific, or have a very limited host-range, and the virions of several different kinds of well-known insect viruses become embedded in crystalline matrices of protein, "polyhedra", which are usually large enough to be seen easily by light microscopy. These embedded viruses are peculiar to insects, mostly to the larvae of Lepidoptera (Fig. 39c), and there are very many known examples. Comparatively few viruses that have non-embedded virions, resembling the kinds that attack most other animals and plants, have so far been identified in insects. A large proportion of them occur in the honey bee (Figs 5, 35; Table I).

I. PARALYSIS

A. *Symptoms*

This virus disease has two distinct sets of symptoms, or syndromes (Bailey, 1975). One of these (Type 1), seemingly the commonest in Britain and described by beekeepers as "paralysis" more than a hundred years ago, includes an abnormal trembling motion of the wings and bodies of affected

bees. These fail to fly but often crawl on the ground and up grass stems, sometimes in masses of thousands of individuals. Frequently they huddle together on top of the cluster in the hive. They often have bloated abdomens and partially spread, dislocated wings (Fig. 36b). The bloated abdomen is caused by distension of the honey sac with liquid (Fig. 36d). The mechanical effect of this accelerates the onset of so-called "dysentery" (Fig. 41f), and sick individuals die within a few days. Severely affected colonies suddenly collapse, often within a week and particularly at the height of summer, leaving the queen with a handful of bees on neglected combs (Bailey, 1969b). All these signs are the same as those that were attributed to the "Isle of Wight disease" (Chapter 9, V.). This kind of paralysis also seems to correspond to

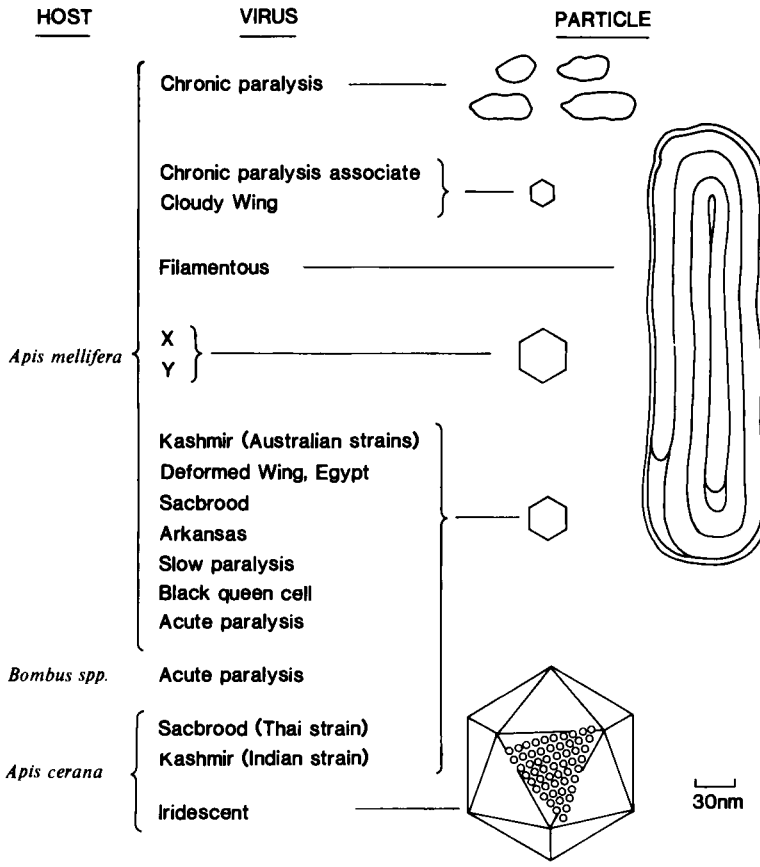

Figure 5 List and diagrammatic outlines of particles of viruses that attack honey bees.

Table I Properties of honey bee viruses.*

Virus	Dimensions (nm)	S_{20w} (Svedbergs)	Buoyant density in CsCl (g/ml)	Nucleic acid		Proteins
				Type	Molecular wts ($\times 10^{-6}$)	Molecular wts ($\times 10^{-3}$)
Chronic paralysis	20 × 30 to 60	80 to 130	1.33	RNA	1.35, 0.9, 0.35†	23.5
Chronic paralysis virus associate	17	41	1.38	RNA	0.35†	15
Cloudy wing	17	49	1.38	RNA	0.45	19
Acute paralysis	30	160	1.37	RNA	N.D.	24, 33, 35‡
Arkansas	30	128	1.37	RNA	1.8	41
Black queen cell	30	151	1.34	RNA	2.8	6,29,32,34
Deformed wing	30	165	1.37	RNA	N.D.	N.D.
Egypt	30	165	1.37	RNA	N.D.	25,30,41
Kashmir (India strain: (*Apis cerana*)	30	172	1.37	RNA	N.D.	24,37,41
Kashmir (Australian strains)	30	172	1.37	RNA	N.D.	25,33,36,40,44
Sacbrood	30	160	1.35	RNA	2.8	26,28,31
Sacbrood (Thai strain: *Apis cerana*)	30	160	1.35	RNA	2.8	30,34,39
Slow paralysis	30	176	1.37	RNA	N.D.	27,29,46
X	35	187	1.35	RNA	N.D.	52
Y	35	187	1.35	RNA	N.D.	50
Iridescent (*Apis cerana*)	150	2216	1.32	DNA	N.D.	N.D.
Filamentous	150 × 450	N.D.	1.28	DNA	12.0	13 to 70¶

* Mostly from Bailey (1976), or original; † Overton *et al.* (1982); ‡ Ball (1985); ¶ About 12 proteins, Bailey *et al.* (1981b).
N.D. = not determined.

the disease long known in Europe as *Waldtrachkrankheit*, so named because it often seems to be associated with nectar gathered from the forests.

The other syndrome (Type 2) has been given a variety of names: "black robbers" and "little blacks" in Britain, *Schwarzsucht* and *mal noir* or *mal nero* in continental Europe; and could well have been the condition described by Aristotle of a black bee with a broad abdomen which he called "a thief" (φώρ). At first the affected bees can fly, but they become almost hairless, appearing dark or almost black which makes them seem smaller than usual but with a relatively broad abdomen; they are shiny, appearing greasy in bright light (Fig. 36c). They suffer nibbling attacks by other bees in the colony, which may account for their hairlessness, and, when they fly, they are hindered from returning to their colony by the guard bees, which makes them seem like robber bees (Drum and Rothenbuhler, 1983). In a few days they become trembly and flightless and soon die. Both syndromes often occur in one colony, but usually one or the other predominates.

Sections of the hind-gut epithelium of paralytic bees show basophilic cytoplasmic bodies (Fig. 36f), which seem to be specific to the disease and were first described by Morison (1936) who suspected they were associated with a virus.

B. *Cause*

The virus that causes paralysis (Figs 5, 35b), is called chronic paralysis virus to distinguish it from acute paralysis virus (Section III) which was found at the same time (Bailey, 1976). The properties of chronic paralysis virus particles are given in Table I. When injected into, fed to, or sprayed on adult bees, purified preparations of the particles cause paralysis, usually with the Type 1 syndrome. The difference between the syndromes probably expresses genetic differences between individual bees: there is considerable evidence that susceptibility to the multiplication of chronic paralysis virus is closely limited by several inherited qualities of bees and some variation of these qualities might well lead to variations in the symptoms. Rinderer *et al.* (1975) and Kulinćević and Rothenbuhler (1975) were able to select strains of bees which were more susceptible than usual to a "hairless black syndrome", later shown to be chronic paralysis by Rinderer and Green (1976). Other circumstantial evidence indicating that susceptibility to paralysis is closely limited by hereditary factors has been discussed by Bailey (1965a, 1967d). Inbreeding with colonies that have paralysis, or allowing them to rear their own queens that mate with drones from similar colonies, maintains a higher incidence of the disease than when they are supplied with queens from elsewhere.

C. *Multiplication and Spread*

Many millions of particles of chronic paralysis virus can be extracted from one bee with paralysis. Many tissues become infected with the virus, including the brain and nerve ganglia. Occasionally pupae are killed by the virus at a late stage in their development in colonies suffering severely from paralysis. In the laboratory the virus multiplies more in bees kept at 30°C than at 35°C, but it kills bees quickest at the higher temperature.

Very many millions of particles are needed to infect a bee by mouth and cause paralysis, but about 100 or fewer will cause the disease when injected into the haemolymph. The sensitivity of bees to ingested virus is increased somewhat by the admixture of broken hairs (Rinderer and Rothenbuhler, 1975). Another likely method of infection in nature, which requires only few particles, is via pores in the cuticle left by broken bristles (Bailey *et al.*, 1983a). This briefly exposes the cytoplasm of epithelial tissue, and when bees are crowded together virus can become rubbed into the wound.

Much chronic paralysis virus is in the distended honey sacs of paralytic bees and in the pollen collected by apparently normal individuals from colonies suffering from paralysis. The virus is probably secreted by the bees from their food glands into the liquid that enters the honey sac, which is then added to the pollen they collect (Bailey, 1976). Perhaps of greater significance is the fact that chronic paralysis virus occurs commonly in colonies that are accepted by beekeepers as healthy. Sensitive infectivity tests have shown that apparently normal live bees often contain some of the virus. There is no particular time of year when paralysis, or the virus in seemingly healthy colonies, becomes most common. Therefore, irregular factors such as poor weather or crop failure or certain beekeeping activities, which quickly suppress the activity of bees, rather than seasonal events may largely determine the rate at which it spreads between bees. The unusual crowding of bees within the colony, which occurs for a variety of such reasons, both natural and artificial, and a consequent increase of transmission of virus via the pores left in the cuticle by broken bristles and by the ingestion of these bristles, would be compatible with the irregularities of infection and of outbreaks of paralysis. Kulinćević *et al.* (1973) observed that symptoms of paralysis occurred sooner in bees when they were deprived of their queen. Such bees decrease foraging and also become agitated, so perhaps suffering more physical damage than usual within the colony.

D. *Occurrence*

Chronic paralysis virus has been detected serologically in extracts of bees found with paralysis symptoms in Australia, New Zealand, China, Mexico,

USA, Scandinavia, continental Europe, the Mediterranean area and many parts of Britain; and virus particles with the same appearance have been described occurring in the Ukraine, France and Canada. Infectivity tests with extracts of bees from apparently normal colonies in Britain have shown that the virus is commonly distributed among them throughout the year and causes mortality that sometimes approaches 30% of the total usually accepted as normal (Bailey, 1976; Bailey *et al.*, 1981a).

E. *Changing Incidence in Britain*

The incidence of chronic bee paralysis declined in Britain from about 8% of samples submitted by beekeepers, when records began in 1947 (Anon, 1947–1980), to less than 2% by 1963 (Fig. 6). The rate of decrease was very

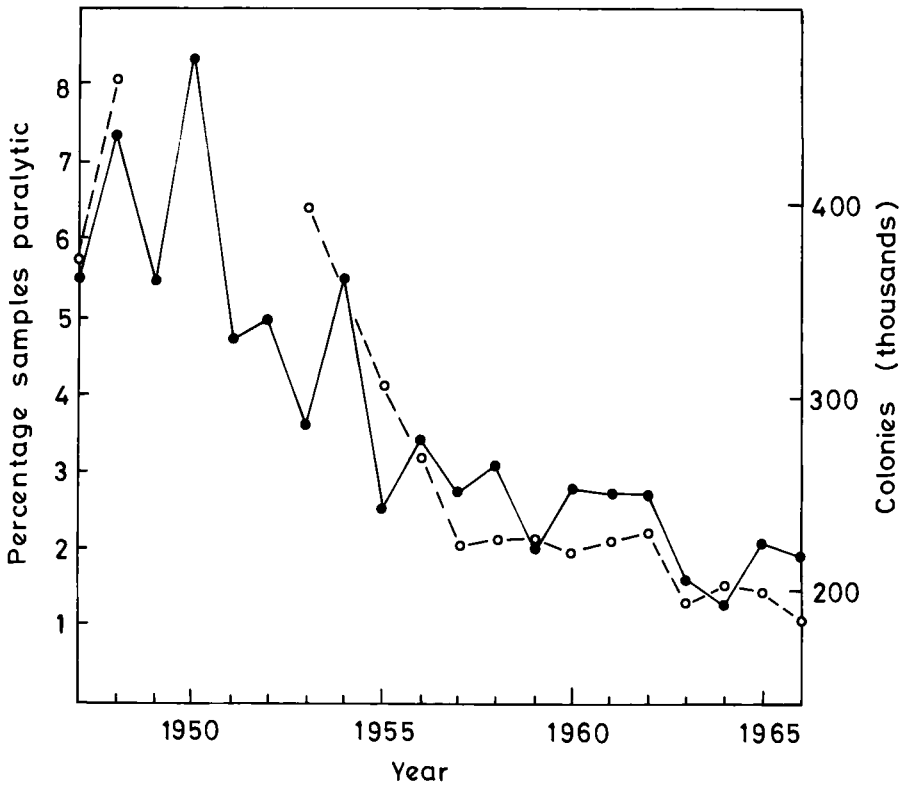

Figure 6 The percentages of samples of adult bees with paralysis (•) in England and Wales, and the total numbers of bee colonies (○) from 1947 to 1966. (From Bailey *et al.*, 1983a.)

closely and significantly associated with that of the number of colonies of bees in Britain. Exactly the same significant regression on the numbers of colonies occurred during the same period with infestation by *A. woodi* (Chapter 7, I.E.; Fig. 29). This parasite is also widespread and enzootic, but is independent of paralysis and causes no overt signs of infestation.

The significant regression of infestation by *A. woodi* on colony numbers in Britain, together with previous records for *A. woodi* (Morison *et al.*, 1956; Fig. 29) strongly suggests there were more colonies, or higher local concentrations of them, or both, during the early part of this century than since 1947, i.e. on average, the country was more overpopulated with bees than it is today. The aggravating effect of overpopulation on infestation by *A. woodi* is discussed later (Chapter 7, I.C.E.; 9, V.), and the same applies to bee paralysis: the virus spreads within colonies by close contact between live individual bees, as discussed above (Section D.). Relevant to this point, there is much paralysis today in the Black Forest region of Germany (Ball and Allen, 1988) where the population density of bee colonies is considerably higher than the already high national average (Fig. 43).

The data given in Figs 6 and 29 strongly suggest that about 25% of colonies, possibly more during poor seasons, suffered visibly from paralysis in Britain during the early 1900s. This would have been very impressive and may well have formed much of the core of opinion at the time that a virulent infectious disease was killing numerous adult bees and colonies. There are no data from those early days, but Raymond Bush (1949), a well-known professional fruit farmer, gives a graphic and entertaining first-hand account of the disease during the 1915–1920 period ("summer came and soon the Isle of Wight disease") and of the dramatic curative effect of low colony densities and good nectar-flows.

F. *Chronic Bee-paralysis Virus Associate*

A virus-like particle, 17 nm across (Figs 5, 35a) is consistently associated with chronic bee-paralysis virus but is serologically unrelated to this virus. It does not multiply when injected alone into bees, and therefore may be a satellite of the paralysis virus, depending on it in the way that similar small particles occurring in plants and animals need genetic information supplied by other viruses in order to replicate. As with these satellites and their helper-viruses, the associate particle interferes with the multiplication of chronic paralysis virus in individual bees, inhibiting particularly the relative amount of the longest, most infective particles (Ball *et al.*, 1985). It is more evident in queens than in workers (Bailey *et al.*, 1980a) and may be of some significance in, or a reflection of, the defence mechanisms of individuals against paralysis.

II. SACBROOD

A. *Symptoms*

Whereas healthy bee larvae pupate 4 days after they have been sealed in their cells, larvae with sacbrood fail to pupate, and remain stretched on their backs with their heads toward the cell capping. Fluid then accumulates between the body of a diseased larva and its tough unshed skin (Fig. 36), and the body colour of the larva changes from pearly white to a pale yellow. After it has died a few days later, it becomes dark brown. The head and thoracic regions darken first and, at this stage (Fig. 36i), the signs are most distinctive and specific. Finally, the larva dries down to a flattened gondola-shaped scale.

B. *Cause*

The properties of sacbrood virus particles are given in Table I. When added to the food of unsealed larvae in bee colonies, the larvae die of sacbrood shortly after they have been sealed in their cells. Larvae about 2 days old are most susceptible.

C. *Multiplication and Spread*

Sacbrood virus multiplies in several body tissues of young larvae but they continue to appear normal until after they are sealed in their cells. Then they are unable to shed their last larval skin, because the thick tough endocuticle remains undissolved, and they die. Presumably, infection prevents the usual formation of chitinase by damaging the dermal glands. Each larva killed by sacbrood contains about a milligram of sacbrood virus, enough to infect every larva in more than a 1000 colonies. Yet, in natural circumstances, sacbrood usually remains slight, and usually abates markedly and spontaneously during the late summer. This is because adult bees detect many larvae in the early stages of sacbrood and remove them from the bee colony, and because the virus quickly loses infectivity in the dried remains of those that are left.

Continuity of infection from year to year is provided by adult bees in which sacbrood virus multiplies without causing obvious disease. The youngest workers are the most susceptible and probably become infected in nature mostly when they remove larvae killed by sacbrood. During this activity, they ingest liquid constituents, especially the virus-laden ecdysial fluid (Bailey, 1967d) of larvae that become damaged in the process. Within a day after young bees ingest such material, much sacbrood virus begins to collect in their hypopharyngeal glands (Bailey, 1969a). Infected nurse bees probably transmit sacbrood virus when they

feed larvae with secretions from these glands. Larvae older than about 2 days survive after ingesting the virus and some of these seem to be inapparently infected when they become adults (Anderson and Gibbs, 1989).

However, infected adult bees either cannot be very efficient vectors or they must usually be prevented from transmitting much virus, otherwise sacbrood would not subside spontaneously in summer. Much evidence shows that they are usually prevented from transmitting the virus by behavioural changes (Bailey and Fernando, 1972). Their hypopharyngeal glands degenerate (Du and Zhang, 1985), and they cease to eat pollen and soon cease to feed and tend larvae. They fly and forage, but do so much earlier in life than usual, and they almost all fail to collect pollen (Table II). The few that do collect pollen add much sacbrood virus to their pollen loads, probably in the gland secretions they add to pollen as they collect it. Were many infected bees to tend larvae and later gather pollen, which is quickly consumed by young susceptible individuals, the virus would soon reach and kill more larvae before losing its infectivity. Sacbrood virus put into nectar gathered by infected bees is a far less important source of infection because the incoming nectar is quickly and widely distributed and diluted within the bee colony where the virus soon loses infectivity, whereas pollen loads remain intact and are usually placed near the brood. Any virus in them remains concentrated and more likely to infect a young nurse bee. Transmission of sacbrood virus from infected adults to larvae is most likely during periods when the division of labour of bees is least well developed, such as the early part of the year or prolonged periods of dearth.

Interestingly, exactly the same permanent changes in behaviour occur in young worker bees that are briefly anaesthetized with CO_2 or other forms of anoxia (Ribbands, 1953), as occur in those infected with sacbrood virus, including a permanent loss of appetite for pollen (Bailey, 1969a). They are equivalent to the changes that occur with age in healthy bees, and the same

Table II Numbers of marked bees seen foraging, and (in parenthesis) the percentage collecting pollen, after equal numbers were infected with sacbrood virus or left untreated when 5 days old (after Bailey and Fernando, 1972).

Days after infection	Treatment	
	Infected	Nil
1–5	64 (3.1)	0
6–10	140 (0.7)	58 (3.4)
11–15	14 (0)	229 (17.0)
16–20	0	46 (10.8)
21–25	0	0

mechanism may be activated by both sacbrood virus and CO_2. It may well be a response to acidosis caused by CO_2 or following damage to oxidative processes in tissues caused by ageing, or by sacbrood virus.

Accompanying the behavioural changes, the metabolic rate of infected workers is diminished and their lives are shortened to about the same length as healthy workers that are completely deprived of pollen. These effects of sacbrood virus further decrease its chances of spread and of surviving the winter when infected bees are most likely to become chilled and lost from the cluster. The lives of drones, which do not eat pollen, are seemingly unaffected by the virus, although remarkable quantities of sacbrood virus multiply in their brains.

D. *Occurrence*

Sacbrood was first identified by White (1917) in the USA and shown by him to be caused by a filterable agent. It is now known to be widely distributed throughout the world (Bradbear, 1988). Its reported absence from certain areas, notably large parts of S. America, Africa, the Middle East, Japan and the Malay Archipelago must be viewed with caution. Sacbrood virus is the commonest known bee virus in Eastern Australia (Hornitzky, 1987) occurring in about 90% of colonies in New South Wales and Queensland (Anderson, 1983); and it is extremely common in Britain, although it was believed not to occur there until first identified in 1964 (Bailey, 1975). Before then the disease was believed to be a non-infectious hereditary fault known as "addled brood", because experimenters had failed to spread the disease by placing combs containing diseased larvae in healthy colonies. However, it does not spread readily this way (Section II.C.). Recent surveys in England and Wales show that most colonies are infected and, although most show no signs, up to 30% usually contain a few larvae with sacbrood. Dall (1985) detected the virus in seemingly healthy pupae from bee colonies in South Australia and New South Wales.

A strain of sacbrood virus has been isolated from larvae of *Apis cerana* from Thailand. It is closely related to sacbrood virus of *Apis mellifera*, but has distinctive properties (Tables I, VII; Bailey *et al.*, 1982).

III. ACUTE BEE-PARALYSIS VIRUS

This virus was discovered as a laboratory phenomenon during work on chronic bee-paralysis virus (Bailey *et al.*, 1963). Extracts of chronically paralysed or of seemingly healthy bees were injected into further healthy bees and some

of these were killed by acute paralysis virus. In the laboratory the virus multiplies more in bees kept at 35°C than at 30°C, but it kills the bees quickest at the lower temperature. These effects of temperature are opposite to those applying to chronic paralysis virus.

Further investigations showed that acute paralysis occurs commonly in seemingly healthy bees in Britain, especially during the active season (Fig. 7), but, again in Britain, it has never been associated with disease or mortality of bees in nature. Usually, it appears to be contained within tissues that are not immediately essential to the life of the bee. This contrasts with findings in mainland Europe where acute paralysis virus has been identified as a major cause of adult bee and brood mortality in honey bee colonies severely infested with *Varroa jacobsoni* (Chapter 7, III.; Ball and Allen, 1988). Much acute paralysis virus has also been detected in dead adult bees from colonies infested with *V. jacobsoni* in Florida.

Apparently the mites activate the virus or release it from the tissues in which it is usually contained when they pierce the body wall of inapparently infected bees, which soon become systemically infected and die. The mite also acts as a vector of the virus transmitting it from infected to healthy adult bees as Batuev (1979) demonstrated in laboratory experiments, and to pupae (Ball and Allen, 1988).

Acarapis spp. (Chapter 7) are the only other known ectoparasites of bees that might be expected similarly to transmit the virus, but they do not, at least not in Britain, and probably not elsewhere. The presence of acute paralysis virus in bees sent to Rothamsted from Belize in sufficient amounts to have caused their death (Bailey *et al.*, 1979) suggests strongly that *V. jacobsoni* was in their colonies.

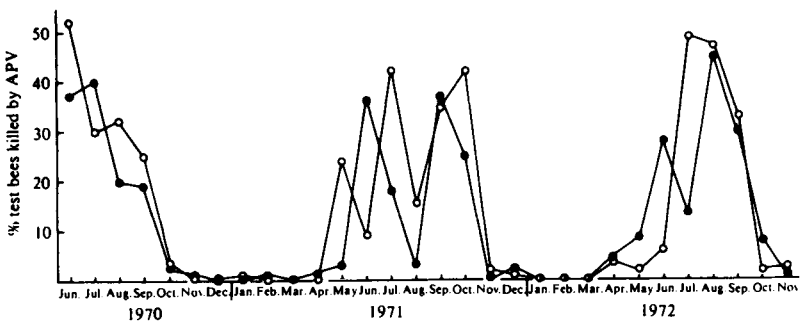

Figure 7 Mean percentage of test bees killed by acute paralysis virus when injected with extracts each of 20 live seemingly healthy adults from each of 2 normal colonies at Rothamsted at 1 site (●) and 2 or 3 colonies at another (○). (From Bailey *et al.*, 1981a.)

Adult bees, in which the virus has been activated or injected by *V. jacobsoni*, can, before they die, infect young larvae, probably by adding much virus to their food in gland secretions (Ball and Allen, 1988). Larvae fed sufficient virus die before they are sealed in their cells; those that survive continue to develop but may emerge as inapparently infected adults.

Acute paralysis virus sometimes occurs in the pollen loads of seemingly healthy foraging bees and in their thoracic salivary glands. It occurs similarly in bumble-bees. It was not found in the pollen of plants (*Trifolium pratense*) visited by the bumble-bees that were collecting pollen (Bailey, 1975), so it seems unlikely to be a plant virus.

IV. DEFORMED WING VIRUS

This virus was first isolated from diseased adult individuals of *Apis mellifera* sent to Rothamsted from Japan. The virus has since been detected in *A. mellifera* from most European countries, Saudi Arabia, Iran, Vietnam and Argentina and in *A. mellifera* and *Apis cerana* from China. Deformed wing virus in diseased brood, dead adults and deformed newly emerged honey bees from many countries is associated with infestation of the colonies with *Varroa jacobsoni* (Chapter 7, III.). Laboratory and field studies have shown that the mite transmits the virus in the same way as acute paralysis virus (Section III; Ball, 1989). Deformed wing virus multiplies slowly and pupae infected at the white-eyed stage of development survive to emergence but have deformed or poorly developed wings and soon die (Fig. 42i). Virus isolates show some differences in their coat proteins but all are serologically closely related to each other and distantly related to Egypt bee virus (Section X.C.).

V. BLACK QUEEN CELL, FILAMENTOUS AND Y VIRUSES

These are three common viruses of special interest because they are intimately associated with *Nosema apis* (Bailey *et al.*, 1981a, 1983b).

Black queen cell virus, as its name suggests, is associated with queen cells that develop dark brown to black cell walls. They contain dead propupae or pupae in which are very many particles of the virus. In the early stages, infected pupae have a pale yellow appearance and a tough sac-like skin, resembling propupae that have died of sacbrood. They are most noticeable when many queen cells are being reared together in "queen rearing" (broodless

and queenless) colonies from young larvae that have been "grafted" or inserted into the colonies by the usual techniques of beekeepers (Laidlaw, 1979), and they are most usual in the early part of the season. By contrast with sacbrood virus, black queen cell virus does not readily multiply when ingested by young worker larvae, young worker bees or drones, or when injected into adult workers or drones (Bailey and Woods, 1977) but it is a common infection of adult bees in the field (Fig. 8).

Filamentous virus (Figs 5, 35f, g), which was originally believed to be a rickettsia (Wille, 1967), was identified as a virus in the USA by Clark (1978). It multiplies in fat-body and ovarian tissues of adult bees, and the haemolymph of severely infected bees becomes milky-white with particles, but no other symptoms are known. The incidence of the virus shows a regular and striking annual cycle with a peak about May and a trough about September (Fig. 9).

Bee virus Y occurs frequently in adult bees during the early summer (Fig. 8). Experimentally, it multiplies when given in food to adult bees kept at 30°C but not at 35°C; and it does not multiply at all when injected into bees, so it may be restricted to the gut epithelium. The virus causes no visible signs of infection.

All three of these viruses almost invariably multiply only in those individual

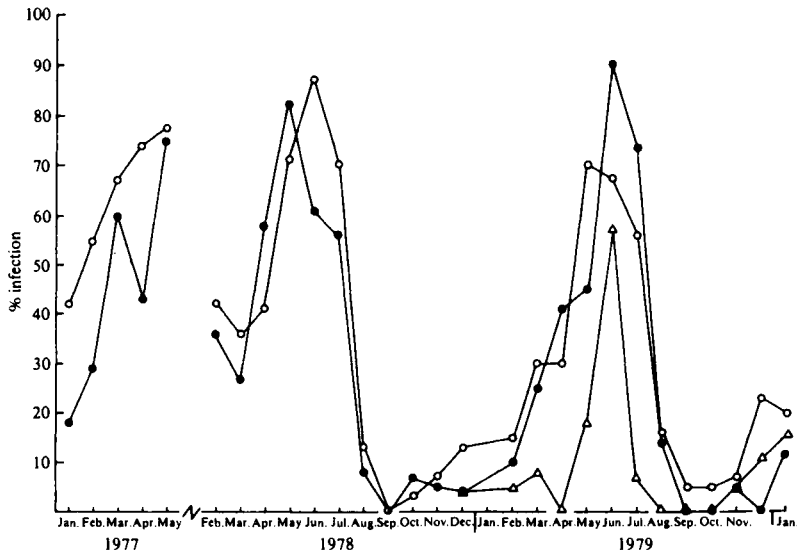

Figure 8 Mean percentage of 25 undisturbed bee colonies at Rothamsted infected with black queen cell virus (●) and bee virus Y (△, not identifiable until December 1978) and of individuals in them infected with *Nosema apis* (○). (From Bailey *et al.*, 1981a.)

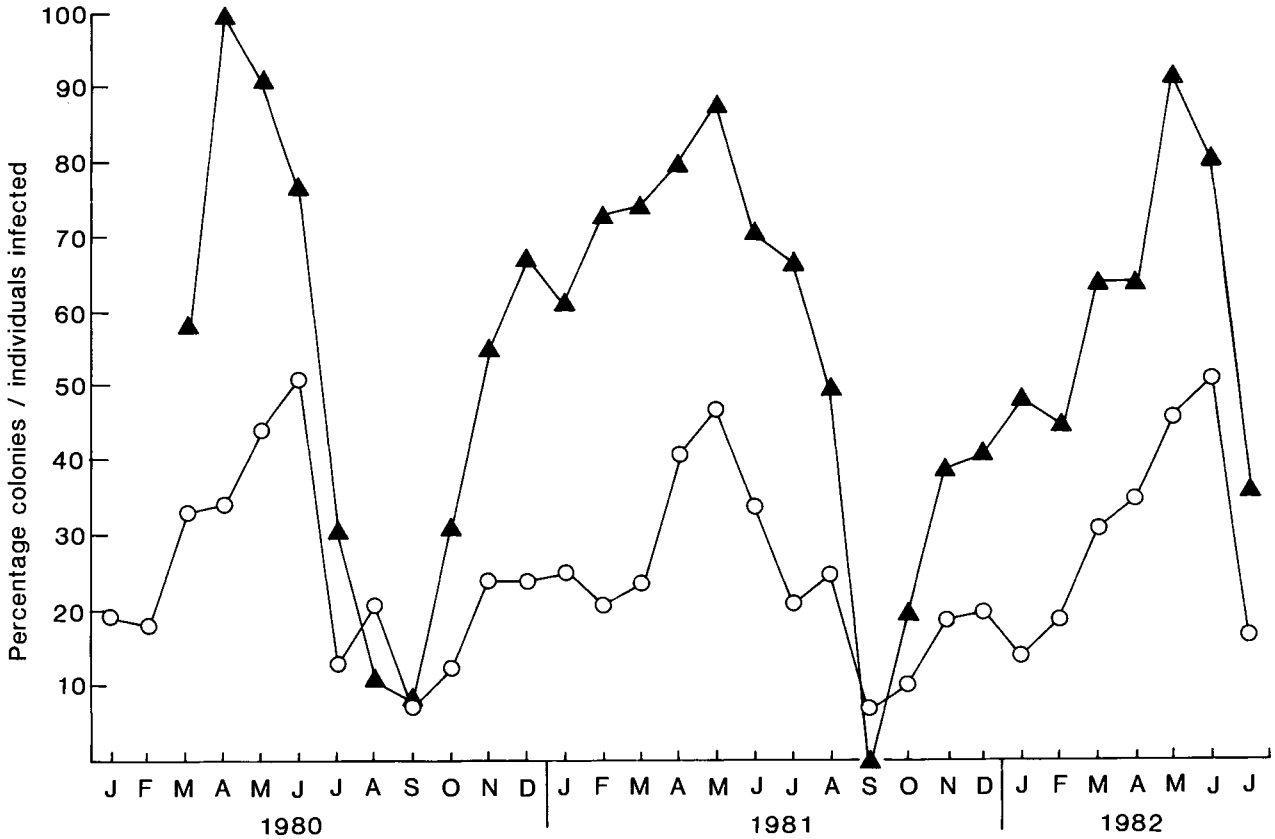

Figure 9 Mean percentage of 25 to 30 bee colonies at Rothamsted infected with filamentous virus (▲), and of individuals infected with *Nosema apis* (○). (From Bailey *et al.*, 1983b.)

Table III Comparative incidence of *Nosema apis* and viruses in 175 bee colonies. (From Bailey *et al.*, 1981a.)

Virus	No. colonies with:		
	N. apis + virus	Virus only	*N. apis* only
Black queen cell	46	1	106
Bee virus Y	38	0	106

adult bees that are also infected with *Nosema apis* (Bailey *et al.*, 1981a) (Tables III and IV). The viruses are unrelated to each other, and seem unlikely to have a common relationship with *N. apis*. A possible reason for their close association with the parasite is that *N. apis* decreases the resistance of bees to infection by viruses that usually invade via the gut. Infection by *N. apis*, which multiplies within the cytoplasm of the cells of the mid-gut epithelium, could be expected to interfere with or prevent the production of a resistance factor, such as the anti-viral protein produced by the gut of silkworms (Hayashiya *et al.*, 1969).

Black queen cell virus and bee virus Y add to the pathogenic effect of *Nosema apis*, and their presence or absence may account for the considerable variations of virulence that have been attributed to the microsporidian, but tests failed to show any similar effect by filamentous virus (Bailey *et al.*, 1983b).

Black queen cell virus, bee virus Y, and filamentous virus have been identified in bees sent to Rothamsted from Britain, N. America and Australia. Filamentous virus has also been found in bees sent from Japan; and Batuev (1980) has reported it in the USSR. All three viruses also occur in mainland Europe (Ball and Allen, 1988).

Table IV Distribution of viruses within dead field bees after separating them into those with and without *Nosema apis* (original data).*

Virus	No. bees examined	Groups or individuals with:		
		N. apis + virus	Virus only	*N. apis* only
Filamentous	32 groups of 10	13	0	0
Black queen cell	6 groups of 30	4	0	0
Y	6 groups of 30	3	0	0
X	452 individuals	28	73	73

VI. BEE VIRUS X

This virus (Figs 5, 35d), distantly related serologically to bee virus Y (Bailey *et al.*, 1980b), occurs in adult bees and, again resembling bee virus Y, has been found to multiply experimentally only when given in food to adult bees kept at 30°C but not in those kept at 35°C; and it does not multiply when injected into them. Yet, unlike bee virus Y, it has no relationship with *Nosema apis* (Table IV), is less common than bee virus Y, and is prevalent at a different time of year from bee virus Y (Bailey *et al.*, 1981a; Fig. 10). Bee virus X shortens the lives of bees significantly when given to them in food.

Bee virus X is associated with *Malpighamoeba mellificae* (Chapter 6, II.) in dead bees in late winter, more frequently than can be expected by chance (Bailey *et al.*, 1983b). This may mean that the virus spreads especially in unusually severe faecal contamination, the same as *M. mellificae* (Chapter 6, II.C.). Bee virus X is not directly dependent on *M. mellificae* in the way that other viruses are dependent on *N. apis* (Section V.) because it multiplies equally well in individual bees in the presence or absence of the protozoan. Its winter epizootic may reflect a low optimum temperature for multiplication.

Bee virus X alone shortens the lives of bees as much or more than *M. mellificae* (Bailey *et al.*, 1983b), so, during its winter epizootic (Fig. 10), it accelerates the death of bees already infected with the protozoan. This curtails the development of many cysts of the protozoan and so diminishes the further distribution of this parasite within the colony in faecal matter, thereby preventing its more usual spring peak of infection (Chapter 6, II.A.) when the colony expands and cleans the contaminated combs (Fig. 20). However, the net result is more harmful to the colony than when the virus is absent because young bees are not produced during the winter to replace losses. *M. mellificae* is invariably blamed when found in colonies that die in late winter or early spring, but the prime cause of death may be infection with bee virus X.

The virus has been found in mainland Europe (Ball and Allen, 1988) as well as Britain.

VII. CLOUDY WING VIRUS

This is a common virus of bees which sometimes show a marked loss of transparency of their wings when they are severely infected. The particles are 17 nm across (Figs 5, 35a) and observations suggest infection is airborne between bees over a short distance (Bailey *et al.*, 1980a). Crystalline arrays of the particles occur between the muscle fibrils (Fig. 39b) to which they may

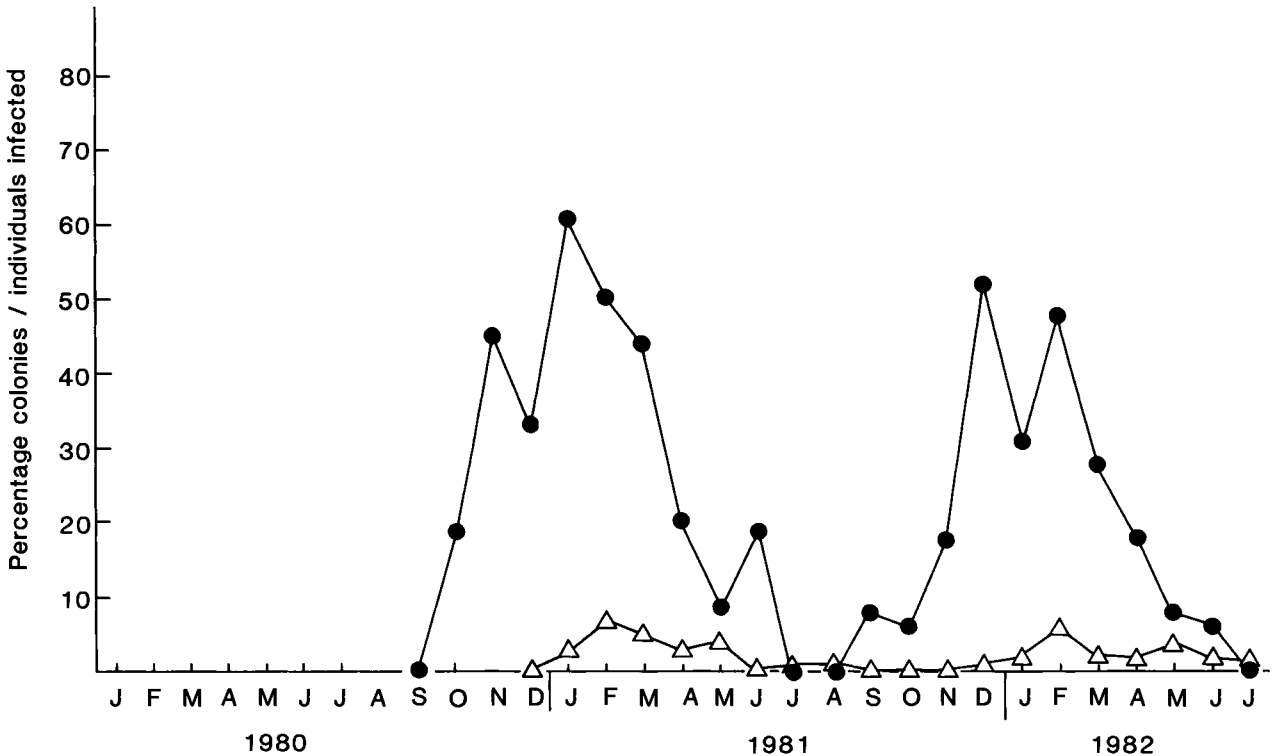

Figure 10 Mean percentage of 25 to 30 bee colonies at Rothamsted infected with bee virus X (●)—especially abundant between September 1980 and July 1982 for unknown reasons—and of individuals infected with *Malpighamoeba mellificae* (△).

be conducted via the tracheal system. Infected individuals soon die.

About 15% of colonies have been found to be infected by the virus in Britain (Bailey *et al.*, 1981a). The virus has also been detected in mainland Europe (Ball and Allen, 1988) and in samples of bees sent to Rothamsted from Egypt and Australia. Some deaths of colonies are associated with severe infection. There is no sign of a seasonal cycle of incidence of infection (Bailey *et al.*, 1983b), which suggests that irregular events determine the rate of spread of the virus, as they do the spread of chronic paralysis virus (Section I.D.E.) and other contagiously transmitted pathogens (Chapter 7, I.C.E.).

VIII. KASHMIR BEE VIRUS

This virus was isolated from diseased adults of *Apis cerana* sent to Rothamsted from Kashmir (Bailey and Woods, 1977) and from Mahableshwar, India (Bailey *et al.*, 1979). It was in company with *Apis* iridescent virus (Section IX.) in the Kashmir bees, but was alone and in large amounts in the bees from India. When injected into adults of *Apis mellifera*, or when rubbed on their bodies, it multiplies quickly and profusely and kills them within 3 days.

Strains of Kashmir bee virus have also been found in adults of *Apis mellifera* in Australia (Bailey *et al.*, 1979) and New Zealand (Anderson, 1985). Three Australian strains of the virus have been identified so far. All are closely related serologically, and they are somewhat less related to the type strain from Kashmir than they are to each other. The Australian strains were associated with severe mortality of adult bees in the field and have also appeared to cause the death of larvae.

There is no evidence that Kashmir bee virus occurs in *Apis mellifera* anywhere other than Australia and New Zealand, and its presence in these countries was unexpected because *A. cerana* does not occur there. Possibly the virus has come from other insect species that are native to both countries and south-east Asia, such as the "sweat" or "stingless" bees of the genus *Trigona*, although Anderson and Gibbs (1982) failed to detect it in 23 colonies of *Trigona* spp. in Australia. Its occurrence in *Apis mellifera* in Australia is a rare example of the recent acquisition of a virus in nature by an insect. The instability of the proteins of the Australian strains of Kashmir bee virus and their serological differences (Bailey *et al.*, 1979) contrast with the stability and uniformity of the other known viruses of *Apis mellifera*. This may reflect a process of mutation and selection that is still being undergone by Kashmir bee virus as it adapts to the honey bee in Australia.

Larvae can survive after they ingest the virus and some of them become inapparently infected adults (Anderson and Gibbs, 1989).

The virulence of Kashmir bee virus and its persistence as an inapparent infection could have severe consequences for beekeeping in Australia and New Zealand were *Varroa jacobsoni* to become established there and if the mite can transmit the virus in the same way that it transmits acute bee-paralysis virus and deformed wing virus (Sections III. and IV.). The same threat would apply to the Americas and Europe were the virus to become established there from imported bees.

IX. *APIS* IRIDESCENT VIRUS

This is the only example of an iridovirus from Hymenoptera and was isolated from adults of *Apis cerana* sent to Rothamsted from Kashmir (Bailey *et al.*, 1976). Iridoviruses are so called because the crystalline masses they form when purified, or even in the tissues where they multiply, appear blue-violet or green when illuminated with bright white light. Many examples are known in a wide range of insects but none are related to *Apis* iridescent virus, although they are physically indistinguishable from it.

Apis iridescent virus is associated with "clustering disease" of *Apis cerana* in India. The most striking and consistent sign of this is an unusual inactivity, especially in summer, of colonies that frequently form small, detached clusters of flightless individuals and often lose many bees crawling on the ground. Large colonies have been said to perish within 2 months of becoming visibly affected (Bailey and Ball, 1978), although Mishra *et al.* (1980) observed that the symptoms subsided when foraging of bees increased.

Iridescent virus-infected tissues can be seen easily by microscopic examination, most clearly in fresh unfixed specimens, but also in bees that are preserved in formalin or alcohol. Infected tissues appear bright blue, in striking contrast to the surrounding creamy white tissues. Single infected cells can be distinguished in otherwise healthy tissue.

Apis iridescent virus has been found only in *Apis cerana* from Kashmir and Northern India, and may be limited in nature to this species in the Himalayan regions, although it can multiply in *Apis mellifera.*

Nothing is known of the natural history of the virus. It multiplies in many different tissues: fat body, alimentary tract, hypopharyngeal glands and ovaries; so it could be transmitted via faeces, eggs, gland secretions or even by ectoparasites. However, it is not transmitted by tracheal mites, such as *Acarapis woodi*, which were first suspected to be the cause of clustering disease, because these were rare or absent in many samples of sick bees, every individual of which was infected with *Apis* iridescent virus (Bailey and Ball, 1978). Curiously, the virus does not multiply when injected into larvae of the greater wax moth

Galleria mellonella, whereas many of the iridescent viruses from other insects multiply readily in wax moths.

X. OTHER BEE VIRUSES

The following viruses or virus-like particles found in bees, and shown to be harmful for them in laboratory tests, are not known to be associated with any particular disease or parasite of bees in the field; or they have not been adequately characterized or shown to be infectious for bees.

A. *Slow Paralysis Virus*

This virus has been found occasionally in extracts of adult bees in Britain and causes their death about 12 days after it is injected into their body cavity (Bailey, 1976).

B. *Arkansas Bee Virus*

This virus was found as an inapparent infection of bees in Arkansas (Bailey and Woods, 1974) by injecting apparently healthy bees with extracts of pollen loads trapped from foragers returning to their colonies. The injected bees died after 15–25 days. It has recently been identified, together with chronic paralysis virus, in dead bees collected from dwindling colonies in California and sent to Rothamsted, but it has not been identified anywhere other than in the USA.

Lommel *et al.* (1985) showed that Californian bees and the original isolates of Arkansas bee virus (Bailey and Woods, 1974) contained another particle, 30 nm in diameter and seemingly unrelated to any known bee virus. Whether it multiplies independently of Arkansas bee virus, and what its effects are, remain unclear. It contains RNA (molecular weight 1.4×10^6), and three proteins of molecular weight 32 000, 35 000 and 37 000.

C. *Egypt Bee Virus*

This virus was isolated from adult bees (*A. mellifera*) in Egypt (Bailey *et al.*, 1979) but nothing is known of its natural history. Young pupae injected with the virus die about seven or eight days later, but attempts to propagate the virus in adult bees have failed.

D. *Possible Bee Viruses*

Chen (1981) refers to an acute and destructive virus disease of *Apis cerana* which has been noticed for several years in Guangdong (S.E. China) and called "large larval disease". An isometric virus resembling that of sacbrood virus, but believed not to be the same, seems to be involved.

Clark (1982a) saw entomopoxvirus-like particles (Bellett *et al.*, 1973) in the haemolymph of three *Bombus* spp. in up to 7% of individuals. The salivary glands seem to be the principal site of infection, but attempts to infect honey bees have been inconclusive.

Table V Cultivation of honey bee viruses.

Virus	Instar*	Method of infection†	Incubation period (days)‡
Chronic paralysis virus (CPV)	A	I	7
	P	I	5
Chronic paralysis virus associate (CPVA)	A } (queens)	I	7
	P } (queens)	I	5
Cloudy wing virus (CWV)	A	?	?
Acute paralysis virus (APV)	A	I	5
	P	I	5
Arkansas bee virus (ABV)	A	I	21
	P	I	5
Black queen cell virus (BQCV)	P	I	5
Deformed wing virus (DWV)	P	I	14
Egypt bee virus (EBV)	P	I	8
Kashmir bee virus (KBV)	A	I	3
	P	I	3
Sacbrood virus (SBV)	L	F	7
	P	I	5
Sacbrood virus, Thai strain (TSBV)	P	I	5
Slow paralysis virus (SPV)	A	I	12
	P	I	5
Apis iridescent virus (AIV)	P	I	5
Bee virus X (BVX)	NA	F	30
Bee virus Y (BVY)	NA	F	30
Filamentous virus (FV)	A	?	?

* A = adults in cages at 35°C; P = pupae in Petri dishes at 96% R.H. and 35°C; L = larvae 2 days old, kept in bee colonies until cells sealed, then incubated without adult bees at 35°C. NA = newly emerged adults in cages at 30°C supplied with pollen.

† I = by injection into haemocoele through abdominal intersegmental membrane; F = in food.

‡ Based on infection with minimum infective doses.

XI. CULTIVATION AND PURIFICATION OF BEE VIRUSES

Many bee viruses can be cultivated in the laboratory, either in adult bees or in bee pupae (Table V), for experimental work, as a very sensitive method of detection, and for the production of antisera.

Adult bees are best collected by placing a comb with adhering bees into a suitable box that can be filled with carbon dioxide. The gas is taken from a pressurized cylinder and passed through water, or a large empty vessel, to remove or melt frozen particles of solid carbon dioxide, which are very injurious to bees. The bees quickly become anaesthetized and can be placed in suitable cages (Fig. 34b). They are allowed to recover at about 20°C before they are incubated in a dry atmosphere at 30°C or 35°C. They are anaesthetized again when they are injected. This is best done the next day because bees sometimes soon die after they have been anaesthetized more than once in 24 h.

The methods found suitable for the purification of bee viruses are given in Tables VI and VII. These methods are neither unique nor inflexible, neither is it likely that they cannot be improved. More than one method is suitable for some of the viruses, but some methods are not suitable for certain viruses, as indicated.

Ammonium acetate buffer is better than phosphate for electron microscopy of preparations negatively stained with neutral phosphotungstate or ammonium molybdate, but phosphate buffer is better for serology. Immunodiffusion tests as described by Mansi (1958) can be done with purified virus or with crude extracts made by grinding the head or abdomen of a bee in 0.05 ml of 0.85% saline + 1 drop of diethyl ether in a small conical tube. The agar for the

Table VI Outline of method for extraction and purification of bee viruses.

1. Grind insects in buffer and solvents (a)
2. Filter through cotton or cheese-cloth
3. Clarify by slow-speed centrifugation (b)
4. Sediment virus from supernatant fluid by high-speed centrifugation (c)
5. Resuspend pellet in buffer (d)
6. Clarify by slow-speed centrifugation
7. Repeat 4, 5 and 6 (except for (d)2 and (d)4 below)
8. Centrifuge down 10–40% (W/V) sucrose gradients (e)
9. Locate and remove virus fractions
10. Dialyse virus fractions against buffer
11. Sediment virus by high-speed centrifugation

a, b, c, d, e: see Table VII for details.

Table VII Details of methods for extraction and purification of bee viruses

	Virus*	Special modifications and additional treatments
(a) Extraction fluid		
1. 0.01 M potassium phosphate (P), pH 7.0 + 0.02% diethyldithiocarbamate (DIECA) + 0.1 vol. diethyl ether (E) followed by emulsification with 0.1 vol. carbon tetrachloride (CCl_4)	All except CWV TSBV, EBV and DWV	Nil
	FV	No E or CCl_4
	AIV	No E
2. 0.01 M ammonium acetate (AA), pH 7.0 + 0.02% DIECA	FV	Nil
3. 0.5 M P, pH 8.0 + 0.2% DIECA + 0.1 vol. E followed by emulsification with 0.1 vol. CCl_4	CWV, SBV, DWV	Nil
	TSBV	+ 0.02 M sodium ethylene diaminetetra-acetate (EDTA)
	EBV	1% ascorbic acid + 2% EDTA instead of DIECA
(b) Slow-speed centrifugation		
1. 8000 g for 10 min	All except FV and AIV	Nil
2. 150 g for 10 min	FV, AIV	Nil

(c) High-speed centrifugation		
1. 75 000 g/3.0 h	All except CWV CPVA, FV and AIV	Nil
2. 75 000 g/3.5 h	CWV, CPVA	Nil
3. 30 000 g/30 min	FV, AIV	Nil
(d) Treatment of sedimented virus		
1. Resuspend in 0.01 M P pH 7.0	All except FV, CWV, EBV, TSBV, BVY and DWV	Keep extracts from pupae at 5°C for several hours to a few days and clarify by low-speed centrifugation, also at 5°C (not suitable for CPV)
2. Resuspend in 0.1M AA, pH 7.0	FV	Layer on 50% w/v sucrose and sediment at 75 000 g/3 h; resuspend in 0.1 M AA pH 7.0
3. Resuspend in 0.5M p, pH 8.0	CWV, EBV, DWV	Nil
	TSBV	+ 0.02M EDTA
4. Resuspend in 0.01M P, pH 7.0, add an equal volume of 0.2 M AA, pH 5.0 (not suitable for ABV, EBV or SBV; less suitable for yield but better for purification of CPV from pupae than 1.)	BVY, BVX, BQCV, CPV, APV	Clarify by low-speed centrifugation; sediment virus by high speed centrifugation; resuspend in 0.1 M AA, pH 7.0 or 0.1 M P, pH 8.0
(e) Sucrose gradients		
1. 45 000 g/3 h at 4°C	All except CPVA, CWV and FV	Sucrose in appropriate buffers, as in (d)
2. 45 00 g/4.5 h at 15°C	CPVA, CWV	
3. 10 000 g/30 min at 5°C	FV	

* See Table V for full names

test contains of 0.05M potassium phosphate buffer (pH 7.0) + 0.005M sodium ethylene diaminetetra-acetate (EDTA) + 0.02% sodium azide for all viruses except the Thai strain of sacbrood virus (TSBV) and Arkansas bee virus (ABV). The best agar for TSBV has the same formulation + 1 to 2% NaCl. A suitable agar for ABV is 0.04M sodium borate (pH 7.0) + 0.85% NaCl + 0.02% sodium azide.

A simple indirect enzyme-linked immunosorbent assay (ELISA) has been used to detect and quantify acute paralysis virus and other honey bee viruses in both adult bees and *Varroa jacobsoni* (Allen *et al.*, 1986). The assay is very specific and sensitive, detecting virus in concentrations as low as 3 ng/ml. However immunodiffusion is still probably the best method for detecting virus that has multiplied in individual bees sufficiently to kill them; it is rapid, inexpensive and specific.

4
BACTERIA

Bacteria are unicellular microscopic organisms without a nuclear membrane surrounding their genetic material and also without the other nuclear structures and organelles that are in cells of higher organisms. Accordingly, they are known as "procaryotic" organisms, whereas fungi and more complex organisms are "eucaryotic". Bacteria have cell walls which give them some rigidity and characteristic shapes but the other procaryotic organisms, the mycoplasmas and spiroplasmas, are delimited by a membrane only and, therefore, are more pleomorphic than bacteria. Most bacteria can be cultivated on artificial media and most are beneficial saprophytic organisms. They are ubiquitous and occur in immense numbers and variety, but comparatively few cause disease. There are only four well-known bacteria or bacterial groups that are pathogens of insects, and two of them attack honey bees.

Various strains of *Bacillus thuringiensis* are well-known pathogens that can kill a wide range of the larvae of lepidoptera. The strain that is most pathogenic for the silkworm was identified first, about the beginning of the century. Other strains have shown some promise as agents for controlling wax moths (Chapter 10, VI.A.). Another group of bacilli, of which *Bacillus popilliae* and *Bacillus lentimoribus* are best known, cause "milky disease" of the ground-dwelling larvae of certain beetles.

Many other bacteria occur in insects, but most are commensals, or are secondary invaders in diseased individuals, either as saprophytes or as weak pathogens. Sometimes they are uncommon or of dubious nature.

Similarly in honey bees, there are two well-known and widely distributed bacterial diseases, "American foulbrood" and "European foulbrood", and there are numerous bacteria which are mostly harmless commensals, saprophytes or uncommon pathogens.

I. AMERICAN FOULBROOD

A. *Symptoms and Diagnosis*

American foulbrood is a disease of larvae which almost always kills them after they have spun their cocoons and stretched out on their backs with their heads towards the cell cappings. These are usually propupae but some pupae die too. They then turn brown, putrefy and give off an objectionable fish-glue-like smell. After about a month they dry down to a hard adherent scale (Fig. 37a) (White, 1920a). The average time before an infected larva shows signs of disease is 12.5 days after hatching, with almost all diseased larvae becoming visibly discoloured between 10 and 15 days after hatching (Park, 1953). The cappings over such larvae quickly become moist and dark-coloured; they then sink inwards and adult bees begin to remove them, first forming small holes and finally leaving the cell fully open. When a matchstick is thrust into the larval remains at the sunken capping stage and then removed, it draws out the brown, semi-fluid remains in a ropy thread (Fig. 37c).

Dry scales fluoresce strongly in ultraviolet light which can help diagnosis with badly preserved material. When a dry scale is placed in 6 drops of milk warmed to about 74°C the milk curdles in about 1 min and then begins to clear at once, all the curd dissolving after 15 min. The effect is caused by stable proteolytic enzymes liberated by *B. larvae* (see below) when it sporulates (Holst and Sturtevant, 1940). It is not caused by any material likely to be tested from colonies, other than by scales of larvae that have died of American foulbrood or by stored pollen, which causes curdling and may appear to cause subsequent clearing (Patel and Gochnauer, 1958). A simpler test is to macerate a little suspect material with 2 drops of milk on a glass slide. Most American foulbrood material produces a firm curd in less than 40 s; European foulbrood material takes at least 1 min 47 s, and healthy larvae take at least 13 min. However, scales may give negative results when they have been in combs that have been fumigated with formaldehyde or paradichlorbenzene, and sometimes for unknown reasons. Dead larvae that have not reached the "ropy" stage do not give a positive reaction (Katznelson and Lochhead, 1947).

B. *Cause*

American foulbrood is caused by *Bacillus larvae*, a rod-shaped bacterium about 2.5–5 μm by 0.5–0.8 μm. It is motile with peritrichous flagella and is Gram-positive. It forms oval endospores which measure about 1.3×0.6 μm (Fig. 38h). These are very resistant to heat and chemical disinfectants; and to desiccation for at least 35 years (Haseman, 1961). The bacillus appears to be specifically associated with the honey bee and attacks the larvae of workers,

queens and drones. Microscopic examination of larval remains shows masses of oval spores with no other organisms. "Giant whips", the coalesced flagella of vegetative rods (Fig. 38i), are readily seen under dark-ground illumination or by phase contrast.

C. *Multiplication of* B. larvae

Larvae become infected by swallowing spores that contaminate their food. Millions of spores are required to infect a larva older than 2 days, but larvae up to 24 h become infected with about ten spores or fewer (Woodrow, 1942). Vegetative cells of *B. larvae* are not infective (Tarr, 1937a; Woodrow and Holst, 1942) probably because the food surrounding the young larvae has a bactericidal effect, which Holst (1946) demonstrated on *B. larvae* in artificial culture. The bactericidal effect, which is in part, if not entirely, due to 10-hydroxydecenoic acid (Blum *et al.*, 1959), decreases when the acid food is neutralized in the larval intestine where the pH is about 6.6.

The spores germinate soon after they enter the larval gut, probably stimulated by CO_2 from the tissues, as are spores of *Ascosphaera apis* (Chapter 5, I.C.). Rods, presumed to be vegetative forms of *B. larvae*, were found by Woodrow and Holst (1942) in larval intestines within 24 h of the larvae having their food inoculated with spores. However, they do not multiply in the lumen of the intestine, but eventually penetrate to the haemolymph and then multiply abundantly. Rods were found by Jaekel (1930) in the haemolymph of 2- or 3-day old larvae, but these were probably severely infected and seem to have been moribund, if not dead. The bacteria do not usually penetrate the gut wall until the larva pupates. For example, in other observations, rods were found after 24 h in the gut of day-old larvae infected with moderate numbers of spores. Few were to be seen there, none were elsewhere when the larvae were between 3 and 6 days old and none could be found anywhere in the larvae when they were between 7 and 9 days old; but the tissues of all larvae between 13 and 14 days old were severely infected (Kitaoka *et al.*, 1959).

It seems, therefore, that conditions are optimal in the youngest larvae for germination of spores but soon become unsuitable for vegetative growth. This corresponds with the characters of *B. larvae* grown in culture (Section I.F.). The vegetative rods are unable to multiply much in the larval intestine because it is insufficiently aerobic or bacterial growth quickly makes it so. The rods are motile, however, and many migrate to the gut epithelium, possibly because conditions there are most aerobic (Fig. 38j). Spores seem able to germinate in old larvae, although less readily than in young ones (Bamrick, 1967) and the vegetative rods may not have time to reach the gut epithelium and invade the tissues, before they are evacuated in the faeces along with the gut contents. Larvae of queens are more susceptible to infection than larvae of workers of

the same genotype, which in turn are more susceptible than larvae of drones (Rinderer and Rothenbuhler, 1969).

The bacillus proliferates in the tissues of larvae when they become quiescent before pupation. Infected larvae then quickly die and spores form, mostly in propupae 11 days after hatching. About 2500 million spores form in one individual (Sturtevant, 1932) and they are invariably in pure culture. Secondary organisms are unable to grow probably because of the antibiotic released by *B. larvae* as it sporulates (Holst, 1945).

An agglutinin which precipitated cells of *B. larvae* (Gary *et al.*, 1949) has been found in the haemolymph of both larvae and adults of a colony with American foulbrood. However, it also agglutinated cells of *Bacillus subtilis*. Tests were not made with healthy colonies which may have non-specific agglutinins and other anti-bacterial substances that normally abound in the blood of insects (Wagner, 1961), although laboratory experiments by Gilliam and Jeter (1970) indicated that adult bees injected with spores produced specific agglutinins.

D. *Spread of* B. larvae *within the Colony*

There is no obvious seasonal outbreak of American foulbrood: disease occurs at any time of the year when brood is present and it has the reputation of invariably killing the colony. However, colonies may show some disease and then recover for an indefinite period (White, 1920a), although when more than about 100 cells of dead larvae are seen at any one time the colony is likely to succumb (Woodrow and States, 1943). Spores may be transmitted to larvae by adult bees engaged in cleaning combs, or the larvae that subsequently occupy the same cells may become infected by spores that still remain. However, reinfection of larvae reared immediately in cells that have just previously contained dead larvae is surprisingly infrequent. In one case only 8% became diseased (Woodrow and States, 1943), and in another 19%, mostly in severely diseased colonies. Therefore, spores and remains of dead larvae are removed efficiently by adult bees, and it seems that most infection is transmitted to larvae in other cells. This is probably done by the bees mainly engaged in cell-cleaning but in the process of changing their occupation to brood-rearing.

Infected larvae can be detected by adult bees very soon after infection. In some tests, 10–40% were removed by nurse bees before their cells were sealed over, according to the number of spores (1–105) with which they were infected (Woodrow, 1942). In other tests about 50% were removed later, but before they were 11 days old, i.e. before most of them contained spores (Rothenbuhler, 1958; Woodrow and Holst, 1942).

A variety of tissue cells seems to increase in size, and oenocytes multiply

in infected larvae before they are invaded by bacteria (Jaekel, 1930). Possibly the insecticidal factors found in extracts of larvae killed by *B. larvae* (Patel and Gochnauer, 1959) are released by the multiplying bacilli and cause these and other effects, which are detected by adult bees.

Infection may sometimes be eliminated by the action of adult bees, but it seems unlikely that all spores will be removed from a colony once it has had American foulbrood. Spores remain infective for at least 35 years (Haseman, 1961), and some could easily become lodged in unused parts of the nest or in food stores, which may remain untouched for many years.

Diseased colonies that recover seem to do so best during good nectar-flows (Ibragimov, 1958; Reinhardt, 1947). Out of 14 diseased colonies observed by Ryan and Cunningham (1950) in Tasmania, eight had less disease and four had shown no increase after the main nectar-flow. Spores may be so diluted by the incoming nectar that young susceptible larvae have little chance of receiving them in their food. Bees also avoid cells that contain visible remains of dead larvae when storing their nectar or pollen (Woodrow, 1941b) and nectar flows stimulate the hygienic behaviour of the older bees of colonies that have been selected for the ability of their nurse bees to detect and remove diseased larvae (Thompson, 1964). Pollen collected by bees protects young larvae from infection to some extent when added to their food (Rinderer *et al.*, 1974). This may well happen when pollen is abundant as it usually is during nectar-flows. Moreover, up to 80% of spores that contaminate nectar are removed by the proventriculus of adult bees (Sturtevant and Revell, 1953), so relatively few become lodged in honey. When 500 million spores/day were fed in 1 litre of syrup for 10 days to colonies allegedly susceptible to disease, followed by 5000 million spores/day after a lapse of 20 days for a further 20 days, only 1 or 2 of several thousand individually identified larvae became visibly diseased and only about 10% of the larvae were ejected (Thompson and Rothenbuhler, 1957). Out of 187 samples of commercial honey in the United States only 15 contained spores and only 1 had more than the 50 million spores/litre found necessary to cause disease by feeding (Sturtevant, 1932).

Contrary to popular belief, therefore, the natural rate of spread of infection of *B. larvae* is low, mainly because most spores are removed from circulation by adult bees and because only the youngest larvae are susceptible.

Some, perhaps many, infected colonies survive with little evidence of disease. Hornitzky and Karlovskis (1989) detected *B. larvae* as a contaminant of adult bees in 26% of many colonies that had been without symptoms of American foulbrood for 6 months, and from about 4% of colonies with no history of the disease in Australia. However, when the disease kills a few hundred larvae in a colony the infection then usually spreads quickly and the colony dies.

E. *Occurrence*

American foulbrood occurs in the temperate and sub-tropical regions of all continents, in Japan (Sano, 1982), New Zealand, Hawaii and some of the West Indies; but, according to Bradbear (1988), it has not been found in most of S. America, or in Africa, the Malay Archipelago or the Indian subcontinent, although it was once reported in India (Singh, 1961).

F. *Cultivation of* Bacillus larvae

The easiest method of cultivation, and the most convenient one for diagnostic purposes by routine bacteriological laboratories, is that given by Lloyd (1986). A few drops of the emulsified remains of a larva in a little sterile water are streaked on blood agar (7% sterile defibrinated bovine, ovine or equine blood + Columbia agar base) and incubated at 37°C. Spores germinate well, especially in an atmosphere of 5 to 10% CO_2 (e.g. in a candle jar), and soon give rise to grey colonies of Gram-positive rods. Typical spores of *B. larvae* begin to form after only 2 days. The colonies are catalase-negative by contrast with those of almost all other aerobic spore-forming bacilli. Hornitzky and Karlovskis (1989) describe a very similar method but with a high concentration (7%) of agar which prevents overgrowth by swarming cells of *Bacillus alvei* (Section II.F.).

Apart from this, few media have been found that will induce germination or sporulation. They include extract of larvae (White, 1907); unheated egg yolk (White, 1920a); egg yolk with yeast, carrot extract and peptone (Sturtevant, 1932); and glucose-peptone with thiamine and trace elements (Lochhead, 1942) although it was later found necessary to add soluble starch, or to extract it with activated charcoal to get reliable growth (Foster *et al.*, 1950). Some or all of these media may be improved in an atmosphere of 5 or 10% CO_2, the same as blood agar; but inocula of many millions of spores are usually needed to start growth on them (Tarr, 1937b). However, an inoculum of very few spores will germinate in semi-solid agar of the following medium when it is inoculated while molten and incubated at 34°C: 1% yeast extract ("Difco"), 1% glucose, 1% starch, 0.136% KH_2PO_4, adjusted to pH 6.6 with KOH and autoclaved at 116°C in closed screw-capped tubes for 20 min. The spores germinate between 5 and 10 mm below the surface in a few days and vegetative growth later extends to the surface. (This is analogous to the germination and growth of *Ascosphaera apis* in semi-solid agar (Chapter 5, I.F.), very likely for the same reason. Note, however, that the media are not identical for the two organisms.) The vegetative cells may then be transferred to agar plates of the same medium but without glucose, and sporulation occurs within a few days (Bailey and Lee, 1962).

Virulence or infectivity of *Bacillus larvae* decreases rapidly after it has been cultivated on artificial media (Tarr, 1937a). Some other cultural characters of *B. larvae* are as follows: colonies are whitish, somewhat transparent and slightly glistening; nitrites are usually produced from nitrates (Hitchcock and Wilson, 1973); purine bases are essential for growth; thiamine replaces some of the essential growth factors of vegetable or yeast extracts; acid but no gas is produced from xylose, glucose, fructose, galactose, salicin and sometimes from lactose and sucrose; acid and curdling form in carrot-milk; and carrot-gelatin is slowly liquified (Claus and Berkeley, 1986).

II. EUROPEAN FOULBROOD

A. *Symptoms and Diagnosis*

European foulbrood is a disease of larvae (Fig. 40) which kills them usually when they are 4 or 5 days old, mostly in early summer when colonies are growing rapidly. There is often a well-defined seasonal outbreak followed immediately by a spontaneous recovery (Morgenthaler, 1944; White, 1920b) (Fig. 11). The sick larvae first become displaced in their cells, just as they do when they are deprived of sufficient numbers of adult bees to feed them adequately. They then soon die, become flaccid, turn brown and decompose, often giving off a foul odour or a sour smell, but sometimes having little or no smell.

Before they decompose, diseased or dead larvae can be dissected easily on a microscope slide by grasping the cuticle at the centre of the body with two pairs of forceps which are then pulled apart. The mid-gut contents are left exposed on the slide still within the transparent gelatinous peritrophic "membrane" which is partially, or completely, filled with bacteria in opaque chalk-white clumps. The contents of the mid-guts of healthy larvae, which are less easily dissected, appear golden-brown.

B. *Cause*

European foulbrood is caused by *Melissococcus pluton*, which is a Gram-positive bacterium, lanceolate in shape and occurring singly, or in chains of varying lengths or in clusters (Fig. 38a,c). Its presence was suspected many years ago, and it was then called *Bacillus pluton* (White, 1912, 1920b). When first isolated it was called *Streptococcus pluton* (Bailey, 1957) because of its morphology, and because it did not form spores, which are characteristics of the genus *Bacillus*. Recently, the guanine + cytosine content of its nucleic acid has been found

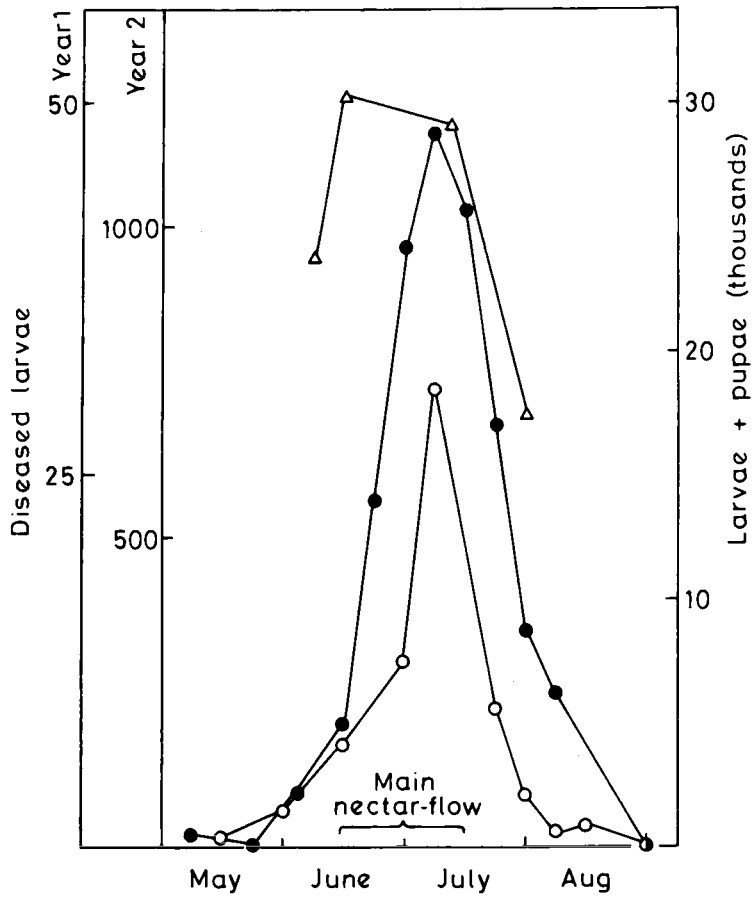

Figure 11 Natural outbreaks of European foulbrood in undisturbed, untreated, naturally infected colonies. Average numbers of diseased larvae in two colonies during Year 1 (●—●) and the average total number of larvae and pupae in them (△—△); and the average number of diseased larvae in four colonies at the same location in Year 2 (○—○). (From Bailey, 1960.)

to be about 29% (Bailey and Collins, 1982a), which excludes it from the genus *Streptococcus*, as now defined (Hardie, 1986), and the organism has become the type species of a new genus (Bailey and Collins, 1982b, 1983).

C. *Multiplication and Spread of* M. Pluton *within the Colony*

The bacteria are swallowed with contaminated food and, as they are microaerophilic to anaerobic and require CO_2 (Section F.), they multiply

vigorously within the food mass in the cavity of the mid-gut, where such conditions prevail (cf. Section I.C.; Chapter 5, C.) and which they sometimes fill almost completely. Infected larvae may nevertheless survive and pupate and the bacteria are then discharged with the faeces and deposited on the walls of the brood-combs cells (Bailey, 1959b). The deposits occur mainly at the base and on the capping of the cell and *M. pluton* in them remains infective, possibly for several years. Most bacteria are removed by house-cleaning adult bees, but some find their way to other larvae.

The infected larvae that survive produce pupae of subnormal weight (Fig. 39), because the bacteria have assimilated much of their food (Bailey, 1960). Larvae are susceptible to infection at any stage of their unsealed life but the older they are the less they are affected, because they lose proportionately less food to the multiplying bacteria. Infected larvae spin feeble cocoons, having poorly developed silk glands, and this encourages the spread of bacteria from their faeces, which otherwise would become sandwiched between layers of cocoon and so removed from circulation.

A balance can exist in an infected colony between the production and dissemination of *M. pluton*, mainly from infected larvae that survive, and its elimination by nurse bees which clean out cells and eject many infected unsealed larvae, often before these are visibly diseased. Infection may persist this way for many years and cause little or no obvious disease. Sensitive serological tests (enzyme-linked immunoabsorbent assays) have detected *M. pluton* in seemingly healthy colonies in Australia at levels that could not be readily detected microscopically (Pinnock and Featherstone, 1984). However, the amount of infection fluctuates, and the fluctuation is related to changing availability of hypopharyngeal protein food from nurse bees. Experiments have shown that infected colonies artificially deprived of much of their unsealed brood keep proportionately more infected larvae than usual (Bailey, 1960). This is because larvae which are left receive surplus food when much brood is removed from colonies (Gontarski, 1953; Simpson, 1958). Conversely, more infected larvae than usual are ejected by bees from colonies with European foulbrood when unsealed brood is added, because infected larvae are the first to show signs of starvation when there is a sudden shortage of glandular food.

In endemically infected colonies in spring, before the seasonal outbreak of disease, there is a balance, as described above, between the increase and spread of *M. pluton* and its elimination by the ejection of infected larvae. When inclement weather or other events interrupt nectar-flows, growth of colonies is checked, and *M. pluton* can then accumulate with little or no sign of disease because of a temporary abundance of nurse bees and of the food they provide both for larvae and bacteria. When the main nectar-flow begins, brood-rearing suddenly increases (Simpson, 1959) and severely infected larvae

then have insufficient glandular food. When this change is rapid, hastened in all probability by the recruitment of young bees to foraging duties, many infected larvae die faster than bees can detect and eject them, thus creating a typical outbreak. Such events led to the detection of European foulbrood for the first time in Africa (Bailey, 1960) when nectar-flows began unusually late in Tanganyika, after prolonged dry weather had restricted brood-rearing. However, the death and ejection of severely infected larvae prevents further transmission of *M. pluton* in their faeces and this, together with the end of colony growth when the nectar-flow ends, usually soon leads to the disappearance of visible disease. The sequence was well illustrated by the abrupt, spontaneous increase and decrease of the numbers of larvae with European foulbrood at the same time of year in the same locality in Britain, during both mild and relatively severe outbreaks of disease, which were coincident with the main nectar-flow and the concomitant peak of brood-rearing (Fig. 11).

Small colonies grow relatively more quickly than large ones in the same favourable conditions, which explains the sudden prevalence of disease often reported among "weak colonies". Prolific queens seem to produce resistant colonies, probably because their brood-nests remain larger in proportion to the number of nurse bees than those of less prolific queens, particularly in adverse circumstances, and so their infected larvae are likely to be detected and ejected. Diseased colonies that are near to starving have been seen to lose all signs of disease (Poltev, 1950), because they eject starving larvae and infected ones are the first to show signs of starvation.

Sometimes colonies are destroyed or seriously crippled by disease when sufficient numbers of *M. pluton* accumulate to kill a large proportion of the brood. The death of many larvae makes more glandular food available for the relatively few remaining larvae, enabling the infected ones of these to produce even more bacteria, and the brood-nest soon becomes choked with decomposing remains. However, most infected colonies are not so severely affected, and they usually end good seasons with surplus honey and no apparent disease. In localities with uninterrupted nectar-flows, in which colonies grow unhindered each year, infection usually remains slight, and its effect on most colonies is transient.

D. *Occurrence*

1. *Apis mellifera*

European foulbrood is widely distributed throughout the main beekeeping areas of the world (Bradbear, 1988). Its reported absence from certain areas, notably parts of S. America and Africa, should be regarded with caution in

view of its comparatively late identification in many parts of the world and of its propensity to persist without causing much or any sign of disease. However, its reported absence from New Zealand, where inspection of bees is good, seems reliable.

Strains of *M. pluton* from all parts of the world are very closely related (Bailey and Gibbs, 1962; Tham, 1978), except one from Brazil which was less closely related to any, including those from other *Apis* species (Section 2.), and can be cultivated on chemically defined media (Bailey, 1984).

2. Other *Apis* species

Several strains of *M. pluton* have been isolated from larvae of *Apis cerana* from India; and one strain was more distantly related to the type strain than the others (Bailey, 1974).

M. pluton has also been isolated from the brood of *Apis laboriosa* (Allen *et al.*, 1990). Colonies of this bee, which is much larger than *A. mellifera* and is closely related to *Apis dorsata*, each build a single large comb, suspended quite exposed from rocks or branches. They cannot be kept in hives but live entirely in the wild. The diseased colonies were found on two cliff faces in the same area of Nepal. Nearly all of 13 colonies were severely diseased, but mortality was predominantly of sealed larvae. *M. pluton* was the only bacterium that could be isolated from the diseased larvae and it was culturally and serologically indistinguishable from the type strain from *A. mellifera*. No viruses were detected and the only other bees in the area were *A. cerana*.

E. *Secondary Bacteria*

The death of infected larvae may be accelerated by secondary bacteria, of which the commonest is "Bacterium eurydice" White. This has its source in the alimentary tract of adult bees and occurs commonly in healthy larvae in small numbers, but is much more numerous in larvae already infected with *M. pluton*. Its incidence is low in winter and early spring, but it becomes abundant in summer. Freshly collected pollen becomes contaminated with it, but the organism dies within a few days in stored pollen. It will multiply in a watery extract of pollen and increases in bees in summer, probably when they are collecting, eating and manipulating pollen (Bailey, 1963b). It takes the form of square-ended rods, single or in chains, or of streptococci, according to its culture medium (Fig. 38e, f), and has often been confused with *M. pluton*. The cultural characteristics of "B. eurydice" have been compared statistically in detail with those of many other bacteria by Jones (1975) who found they most closely resembled those of *Corynebacterium pyogenes*, a pathogen of vertebrates.

A fairly common secondary invader is *Streptococcus faecalis* (*Streptococcus apis* Maassen), which is very like *M. pluton* in appearance (Fig. 38d) and has frequently been confused with it, although the bacteria are serologically and culturally distinct (Bailey and Gibbs, 1962; Gubler, 1954). It does not persist in honey bee colonies by itself, but is brought in by bees from the field where it is very common (Mundt, 1961) and it becomes temporarily established where European foulbrood breaks out. It causes a sour smell, hence the German name, *Sauerbrut*.

Another common secondary organism is *Bacillus alvei* originally believed to cause European foulbrood (Cheshire and Cheyne, 1885), but soon recognized to be a saprophyte living on the dead remains of larvae and not always present (White, 1906). It forms very resistant spores (Fig. 38g) and becomes established in colonies that have had endemic European foulbrood for several years: it causes a characteristic foul odour. *Bacillus laterosporus* also occurs very occasionally in a similar capacity.

Larvae that die decompose rapidly, and many are left by the adult bees to dry to a scale. Only *M. pluton* and spores of *B. alvei* stay alive for long in the scales. Sometimes propupae die after they have voided their gut contents. Few bacteria are left in their remains, but when *B. alvei* is one of them it multiplies quickly and the scale then becomes full of its spores, which are in almost pure culture.

B. alvei has been shown to multiply in propupae killed by sacbrood, after it had been placed in the food of the larvae together with sacbrood virus (Bailey *et al.*, 1973). This was analogous to its behaviour in European foulbrood. It failed to multiply when it was fed alone to the larvae. The secondary organisms described above, and others, have at one time or another been considered pathogenic for larval honey bees and to cause European foulbrood, but without experimental evidence.

The natural history of infection of larvae by *M. pluton* and other organisms is illustrated in Fig. 12. For reasons already discussed, larvae infected with *M. pluton* follow one of four courses of events:

1. They are detected before they are capped and are ejected by nurse bees. *M. pluton* is alone, or is the dominant organism.
2. They die before they are capped and before they are detected by nurse bees. Infection by *M. pluton* is severe and secondary organisms multiply quickly.
3. They are capped and fail to pupate, but usually void most of their intestinal contents. When *Bacillus alvei* is present it multiplies in the remains.
4. They pupate, form normal or undersized adults, and leave infective cells of *M. pluton* in their faecal deposits in the cell.

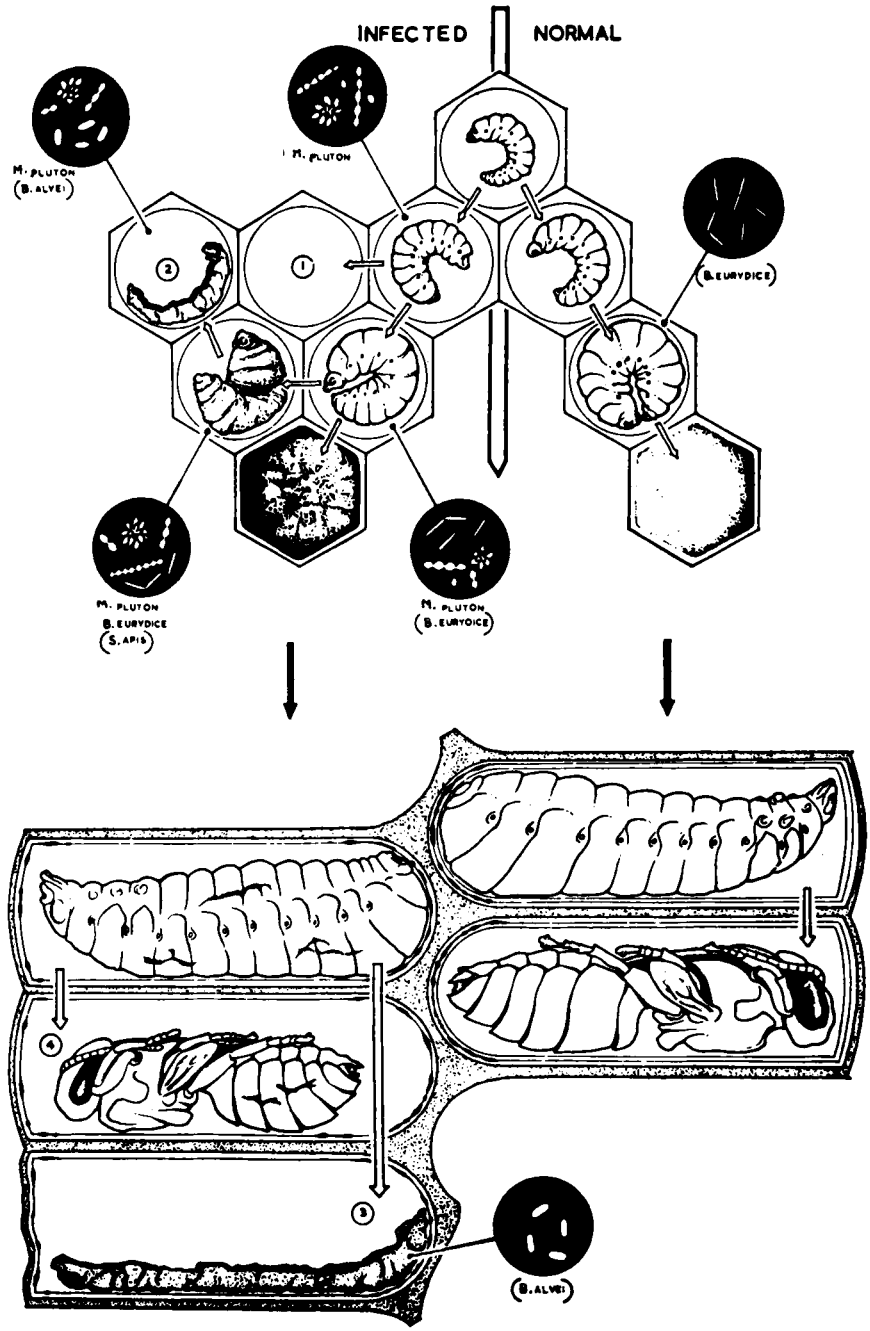

Figure 12 *The natural history of European foulbrood*: Names in the diagram are of dominant bacteria; names in parentheses are of bacteria that do not always occur. See Section II.E. for full explanation.

F. *Cultivation of* Melissococcus pluton

Melissococcus pluton is a microaerophilic to anaerobic organism that requires CO_2 (Bailey, 1957). It may be isolated from diseased or dead larvae or their cell cappings (Section C.), or, ideally, from dry smears of diseased larval midguts (Bailey, 1959c), on a freshly prepared medium composed of yeast extract (most varieties), 1 g; cysteine or cystine, 0.1 g; glucose, 1 g; potassium dihydrogen phosphate, 1.35 g; soluble starch, 1 g. This should be diluted to 100 ml with distilled water, the pH adjusted to 6.6 with KOH, and 2 g agar added and autoclaved at 116°C (10 lb/in^2) for 20 min in closed screw-capped bottles. Fructose may be used instead of glucose but, apart from this or very slight modifications in the proportions of the constituents (Gliński, 1972), no other laboratory conditions are known in which *M. pluton* (except one strain from Brazil (Section D.1.)) will multiply. Certain proprietary peptones, e.g. "Bacto" or "Oxoid", are satisfactory instead of yeast extract, but added cysteine or cystine is essential or much improves growth. Other peptones will not support growth, even with added cysteine or cystine (Bailey and Collins, 1982a).

Watery suspensions of the natural material may be streaked on plates of the special agar, or, preferably, decimal dilutions should be inoculated in the molten agar at 45°C, which is then poured into plates. The plates must then be incubated anaerobically (e.g. in McIntosh and Fildes jars) with about 10% CO_2 at about 34°C. Small white colonies of *M. pluton* usually appear after about 4 days. *M. pluton* is very pleomorphic in culture, often being in rod-like forms (Bailey and Gibbs, 1962).

If the apparatus for anaerobiosis is lacking it is usually easy to cultivate *M. pluton*, when few other organisms are present in the natural material, in deep agar of the special medium. This should be incubated at 34°C, preferably in a closed jar to which some CO_2 is added. When the inoculum is almost free of secondary organisms, particularly *S. faecalis*, growth of *M. pluton* usually occurs below about 8 mm from the surface of the agar.

"B. eurydice" and *B. alvei* grow feebly under the culture conditions necessary for *M. pluton*. *S. faecalis* grows luxuriantly, however, but it can usually be diluted out from natural inocula. Otherwise it produces too much acid, and *M. pluton* cannot grow below a pH of about 5.5.

The presence of much *S. faecalis* or *B. alvei* is presumptive evidence of European foulbrood, but their absence is not evidence that European foulbrood is absent. Both organisms grow well aerobically on ordinary bacteriological media. *S. faecalis* produces small transparent grey colonies within 24 h. Its growth is much stronger with any one of various carbohydrates in the medium, and the final pH is then about 4.0. *B. alvei* grows very quickly and produces a spreading growth of transparent colonies some of which are motile and

move in arcs over the agar surface. The cultures have a characteristic odour and spores form promptly. Further details of the cultural characteristics of these organisms are given in standard works (Claus and Berkeley, 1986; Hardie, 1986).

III. SPIROPLASMAS AND MYCOPLASMAS

A. *Spiroplasmas*

Spiroplasma species are helical motile forms of procaryotes. Several forms are known or suspected causes of certain diseases in plants and insects and the first from insects was isolated from the honey bee (Clark, 1977). This was in the haemolymph of moribund worker bees in Washington DC, and was cultivated on standard media used for *Mycoplasma* species, and also in a mosquito tissue-culture medium. Worker and queen bees were readily infected by feeding or injecting them with the cultured organisms. These investigations led to the discovery of spiroplasmas on plant surfaces and, as a result of this, to the discovery of their widespread occurrence in insects (Clark, 1982b).

In an intensive study of many strains of spiroplasmas, Clark *et al.* (1985) brought together 28 strains isolated from a variety of sources (12 from honey bees; the others from bumble and solitary bees, other insects and the surfaces of flowers) as a new species, *Spiroplasma melliferum*. They are grouped, as sero-group I-2, on the basis of cultural characteristics, nucleic acid and protein types, serology and other features, including having a group-specific lytic virus (SpV4).

In laboratory tests, the spiroplasmas entered the haemocoel of young adult bees 7 to 14 days after they each ingested about 10^6 to 10^7 of the organisms, and they multiplied until there were about 10^{11} per ml of blood before the bees died (Clark, 1982b). They could be seen conveniently by dark-field microscopy of a drop of haemolymph under oil.

Clark *et al.* (1985) and Clark (1982b) consider that the spiroplasmas are deposited on plant surfaces in the faeces of infected bees and other insects in spring. The highest infections (25 to 100%) of bees occurred in May or June in Washington, but they dwindled to nil by mid-July (Clark, 1977). There is no evidence that *Spiroplasma melliferum* multiplies in plants; it seems to be primarily an intestinal infection, where it may not be particularly harmful, of bees and other insects.

Mouches *et al.* (1982, 1984) describe a "May disease" in south-west France that is associated with a spiroplasma of another sero-group (IV) which they call *Spiroplasma apis*. They refer to thousands of dead or moribund, flightless bees with swollen abdomens and agitated movements, many crawling from

their hives in large groups to die about May or June. These are symptoms very like those of Type 1 paralysis (Chapter 3, I.A.) but the abdomens of the French bees were described as "hard" which is not typical of paralysis, and they had many helical spiroplasmas in the blood and digestive tracts. Affected colonies also recovered spontaneously about July, whereas outbreaks of paralysis are irregular and show no seasonal pattern (Chapter 3, I.C.). The spiroplasma was cultivated, and it killed bees about 5 days after it was injected into their haemolymph or about 30 days after they ingested it, unless they had tetracycline added to their food. Mouches *et al.* (1984) isolated serologically indistinguishable strains from flower surfaces in the area where the bees were kept.

The well-defined seasonal peak of incidence of spiroplasmas in the USA and France resembles those of the intestinal parasites *Nosema apis* and *Malpighamoeba mellificae* (Chapter 6, I.C.; II.C.) and suggests that the spiroplasmas could be similarly enzootic in bee colonies. Alternatively they may be enzootic infections of other insects that are prevalent on flowering plants in certain regions and only in spring and early summer. They have not been reported from elsewhere.

Some plant spiroplasmas, e.g. *Spiroplasma citri*, will multiply when injected into bees and kill them, but they seem unable to invade the haemolymph when ingested (Mouches *et al.*, 1982).

B. *Mycoplasmas*

Mycoplasmas are rounded, somewhat amorphous procaryotes and some have been seen by electron microscopy, and photographed, in the ducts of the hypopharyngeal glands of a single 35 day old bee in Brazil (Costa-Leonardo and Silva de Moraes, 1985). The particles, about 1 μm × 2−3 μm, resembled those that have been seen in the insect vectors of mycoplasma diseases of plants, such as "Aster Yellows".

IV. OTHER BACTERIA

Several bacteria have been isolated from adult honey bees, usually from their intestinal tracts (Kluge, 1963), but very little is known of their natural history.

The intenstines of newly emerged bees are free of bacteria; adult bees between 1 and 14 days old, which eat much pollen, have many bacteria in their mid-guts, but later, when their diet is mostly honey, the bacteria almost disappear. However, within the first 2 days of the adult bee's life the pylorus and hind-gut become permanently colonized by bacteria, which grow close

to the surface of the epithelium. In the hind-gut the bacteria are mostly short rods, but in the pylorus there is a variety of forms: cocci, short and long rods, and very long filamentous forms. Some may be forms of "Bacterium eurydice". Drones generally have no bacteria in the pylorus; the few that have are also infected with flagellates (Chapter 6, IV.). Queens, too, usually seem to have no bacteria in the pylorus; only one out of 16 examined was found to have them, and her pylorus was also infected with flagellates (Lotmar, 1946).

Pseudomonas apiseptica and other bacteria have been found in the haemolymph of bees found dying in or near colonies in various parts of North America, Europe and Australia. Bees killed by the bacteria sometimes fall apart at the joints of their bodies. The bacteria grow well on ordinary bacteriological media. Bees are not easily infected when fed with the bacteria or when they are in contact with infected individuals, but they can be more readily infected by brief immersion in a watery suspension of the organisms. Wetting the head and thorax seem more effective than wetting the abdomen (Wille, 1961). This suggests that infection occurs via the tracheae, because inhalation is mostly through the first pair of thoracic spiracles (Bailey, 1954). However, no-one has infected colonies of bees experimentally (Zeitler and Otte, 1967) and naturally occurring disease seems transitory. *P. apiseptica* has been isolated "in abundance" from soil near infected apiaries (Burnside, 1928), and may really be a soil organism occasionally able to infect bees. Its ready growth on ordinary bacteriological media suggests that it is usually a saprophyte and that it may not be specifically associated with honey bees.

Drobńiková (1982) described bees with red-pigmented thoraces and dorsal surfaces of their bodies: they could not fly and soon died of septicaemia caused by *Serratia marcescens*, believed to be synonymous with *P. apiseptica* and which forms a red pigment when it multiplies at about 30°C. Strains of *S. marcescens*, identified as biotype A4(b), were suspected as the cause of death of colonies in the Sudan (El-Sanousi *et al.*, 1987) and pure cultures were pathogenic for bee larvae. However, the impression remains that, in most circumstances, the various agents of septicaemia cause secondary, though fatal infections that are perhaps consequent upon a variety of primary pathogens or non-infectious disorders.

Bacillus pulvifaciens (Katznelson, 1950) seems to cause a rare disease, "powdery scale", of larvae. The larvae form a light brown to yellow, very crumbly scale, but most infected larvae are detected and removed by bees before infective spores form (Hitchcock *et al.*, 1979). The bacterium grows and sporulates on ordinary media and could well be a saprophyte that is a fortuitous and ill-adapted pathogen of bees. Many of its cultural characteristics resemble those of *Bacillus larvae* (Claus and Berkeley, 1986).

Pseudomonas fluorescens, which occurs in soil and water, and *Yersinia pseudotuberculosis*, which can cause septicaemia in humans and other vertebrates,

have been isolated from the haemolymph of bees with symptoms of "Schwarzsucht", a disease that is associated with chronic paralysis virus (Chapter 3, I.A.). The bees were collecting pine honey-dew in Germany and no bacteria could be isolated from the haemolymph of healthy foragers (Horn and Eberspächer, 1976). Cultures of the bacteria fed to caged bees, at the rate of about 1 million organisms per bee, shortened their lives to one or two weeks when the bees were also fed on honey-dew. The effect was diminished when the infected bees were fed on pure sucrose syrup. The bacteria were re-isolated from the haemolymph of sick experimental bees. Honey-dew was found to be less suitable than sucrose syrup as food for healthy bees and was, therefore, considered to make them more susceptible to infection. The possibility was not excluded, however, that bees affected by chronic paralysis virus are much more susceptible than healthy individuals to the very small numbers of these bacteria that they are likely to encounter in nature.

Hafnia alvei was reported by Strick and Madel (1988) to be the most common species of bacterium associated with bees in colonies infested with *Varroa jacobsoni* (Chapter 7, IV.A.; C.4.). The mites transmitted the bacterium, from the cuticle of pupae that had been experimentally contaminated, to their haemolymph, causing septicaemia and death. *H. alvei* is an opportunistic pathogen of man, animals and birds, occurring in soil, sewage and water, and is synonymous with *Bacillus paratyphi alvei* (Sakazaki, 1984), which Bahr (1922) first isolated from the rectal contents of bees in Denmark.

A variety of microbes were seen by Liu and Ritter (1988) on the surface of *Varroa jacobsoni* viewed by scanning electron microscopy. They comprised fungus-like spores and a helical-shaped organism, reminiscent of spiroplasmas (Section III). Sections of the mites showed large colonies of rod-shaped bacteria in the gastric caecae and Malpighian tubules, colonies of amoeboid-like organisms in the rectum, and rickettsia-like organisms in the posterior part of the body cavity.

Wille (1967) identified "rickettsiae", many of which were probably particles of filamentous virus (Chapter 3, V.) according to Clark (1977, 1978), in about 60% of sick or dead colonies in Switzerland. However, Wille observed rickettsia-like particles in company with no other pathogen, including *Nosema apis*, in about 25% of several thousand bees investigated. This suggests that many of the particles were not of the filamentous virus, as this is closely associated with *N. apis* (Bailey *et al.*, 1983b), so they may well have been organisms resembling rickettsiae. True rickettsiae are minute bacteria-like organisms that are obligate, intracellular parasites of the alimentary tract of blood-sucking arthropods.

5
FUNGI

The fungi, or Eumycetes, include the moulds and yeasts and most are saprophytic on decomposing organic matter. However, the first disease of any kind that was correctly diagnosed as being caused by an infectious micro-organism was a fungal disease of an insect. Agostino Bassi showed in 1834 that "calcino" or "muscardine" of the larvae of silkworms was caused by a fungus, known today as *Beauvaria bassiana* (Steinhaus, 1956). This early knowledge was overshadowed by the strong belief that prevailed about spontaneous generation, but after Pasteur (Chapter 1) it soon became clear that many insects were prey to a variety of fungi. However, as with bacteria, many fungi that multiply in insects are saprophytes. These may be secondary invaders of individuals that have been killed or diseased by primary pathogens, which may also be fungi sometimes obscured by the secondary organisms.

I. CHALK-BROOD

A. *Symptoms and Diagnosis*

Larvae die of chalk-brood after their cells have been capped. They are at first somewhat fluffy and swollen, taking on the hexagonal shape of the cell, but later they shrink and become hard. By this stage the cappings have frequently been removed by the bees. Some of the dead larvae remain chalky-white but others become dark blue-grey or almost black (Fig. 40c, d). Drone larvae are frequently more affected than worker larvae, but it is not unusual to find combs with only the worker larvae affected.

Young infected larvae do not usually die or show signs of disease, although many from diseased colonies can be shown to be infected by allowing them to die in the laboratory when their bodies become overgrown with mycelium (Maurizio, 1934). Infected larvae usually die within the first 2 days after they have been sealed in their cells; otherwise they die as propupae. Many of the

cells may remain sealed in severely diseased colonies, and the loose, hard, larval remains then rattle when the comb is shaken.

Pollen which is mouldy with *Bettsia alvei* (Section I.B.) may be mistaken for chalk-brood, but this usually occurs too early in spring to be chalk-brood, and the lumps break up rather easily into fragments representing the original pollen loads.

B. *Cause*

Originally known as *Pericystis apis* but now reclassified and renamed *Ascophaera apis* (Spiltoir, 1955; Spiltoir and Olive, 1955), this fungus infects larvae only and causes chalk-brood. *Ascosphaera apis* is heterothallic, i.e. spores form only where two different strains of mycelium touch each other. Spores form in spherical aggregates within dark brown-green spore cysts ("fruiting bodies") which are about 60 μm in diameter (Fig. 13) and occur on the outer surface of the larval cuticle; they are very resistant and remain infective for at least 15 years (Toumanoff, 1951).

Similar fungi have been found with fruiting bodies, in a single dead pupa of a leaf-cutting bee (Melville and Dade, 1944), and in the tube of a mason bee (Clout, 1956). Such infections are fairly common. Eight species of *Ascosphaera* are now known, but those of the solitary bees are not usually the same as that which attacks honey bees (Skou, 1983; Skou and King, 1984). Nevertheless, *A. apis* can infect the alfalfa leaf-cutting bee (*Megachile rotundata*) under experimental conditions at 30°C (Vandenberg and Stephen, 1983), to cause disease resembling chalk-brood. The usual cause of such a disease in *M. rotundata* is *Ascosphaera aggregata*, but the signs are different, mainly because sporulation occurs beneath the larval cuticle (Skou, 1975). Even so, Rose *et al.* (1984), who examined over 200 of such diseased larvae in 32 samples from 20 areas of the USA and from Canada and Guatemala, recovered *A. apis* from 23%, including every individual of three samples from two areas of the USA. Therefore, although infection was primarily by *A. aggregata*, mixed infections occurred. Rose *et al.* also recovered *A. apis* from one cell of the alkali bee, *Nomia melanderi*; but they recovered *A. apis* only from 260 specimens of chalk-brood from honey bees from the USA, Canada and Central America. All their isolates were tested for compatibility with authentic cultures to establish their identity.

Another variety of *A. apis*, with fruiting bodies about 130 μm across, occurs both in honey bees and solitary bees (Maurizio, 1935). It seems to multiply particularly in larvae killed by European foulbrood, which suggests it may be a saprophyte that acts as a secondary invader of moribund larvae. Its spore cysts are smooth, whereas those of *A. apis* have scattered papillae, visible by scanning electron microscopy (Gochnauer and Hughes, 1976). Skou (1972)

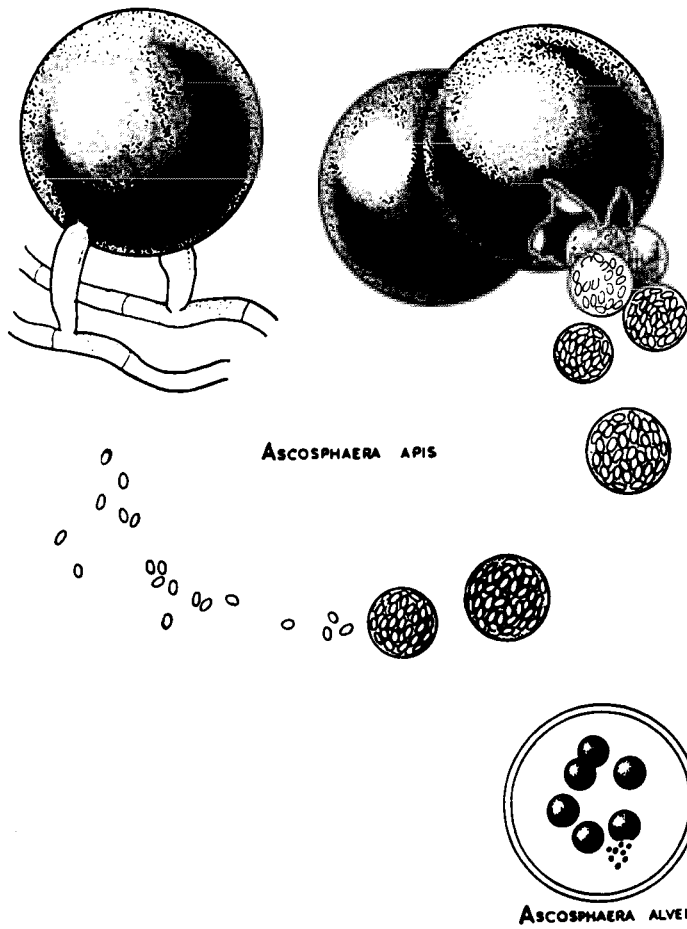

Figure 13 *Ascosphaera apis*. (After Dade, 1949.)

has named it *Ascosphaera major* and refers to it as a cause of chalk-brood equally with *A. apis*, although he isolated it from the leaf-cutter bee, *Megachile centuncularis*. Moreover, he observed that it can multiply on the faecal matter of the bees, which suggests it is primarily a saprophyte. Vandenberg and Stephen (1983) refer to it growing saprophytically on the food in cells of *Megachile rotundata* in laboratory tests but it did not infect the larvae.

Pollen mould, *Ascosphaera alvei* (Betts, 1912), renamed *Bettsia alvei* by Skou (1972), is a fungus rather similar to *Ascosphaera apis* but its spore cysts are only about 30 μm in diameter and its spherical spores are not aggregated

into spore balls. It will grow only at about 18°C and is confined to stored pollen in bee combs.

C. Multiplication

Larvae ingest spores of *A. apis* with their food. The spores germinate in the lumen of the gut, probably activated by CO_2 from the tissues (Heath and Gaze, 1987), and the mycelium begins to grow there, particularly at the hind end (Maurizio, 1934). The mycelium then penetrates the gut wall and eventually breaks out of the hind end of the larva's body, often leaving the head unaffected. When they occur, fruiting bodies form on the outside of the dead larvae.

The nature of the infection is strikingly analogous to that by *Bacillus larvae* (Chapter 4, I.D.). Vegetative growth of *A. apis*, like that of *B. larvae*, is poor in the lumen of the gut of growing larvae, which is insufficiently aerobic, or the mycelial growth quickly makes it so; and *A. apis* depends on the death of its host to form its infective spores. However, *A. apis* grows best in slightly chilled larvae as its optimal temperature for growth and formation of fruiting bodies is about 30°C (Maurizio, 1934). Experiments have shown that brood is most susceptible when chilled immediately after it has been capped (Bailey, 1967b) (Fig. 14). The chilling need be only a slight reduction of temperature, from the normal 35°C to about 30°C, for a few hours; and it can easily occur, even in warm climates, in colonies that temporarily have insufficient adult bees to incubate their brood adequately. Larvae are most likely to be chilled in early summer when colonies are growing, and drone larvae often suffer most as they are generally on the periphery of brood nests. The smallest colonies are at the greatest risk of becoming chilled because they have the lowest capacity for heat and relatively large surface areas. Heath (1982a, b), in extensive reviews, quotes several observations that chalk-brood is aggravated when colonies are rapidly expanding in spring, i.e. when the ratio of brood to adult bees is high, or when it is increased experimentally; and that very small colonies used for mating virgin queens or in observation hives are very susceptible. Koenig *et al.* (1987) also noted that decreasing the ratio of adult bees to brood aggravated chalk-brood; and Pederson (1976) showed that artificially heating hives in spring diminished the incidence of the disease. Other non-lethal factors, such as slight infections by viruses or bacteria, or poisoning, or inadequate food from diseased nurse bees may well cause the same effect as chilling by slowing the rate of development of larvae.

The increasing susceptibility of larvae with age (Fig. 14) contrasts with their decreasing susceptibility to *Bacillus larvae* (Chapter 4, I.C.). Presumably the fungal spores germinate in young larvae but most of the mycelia must then lose their invasiveness.

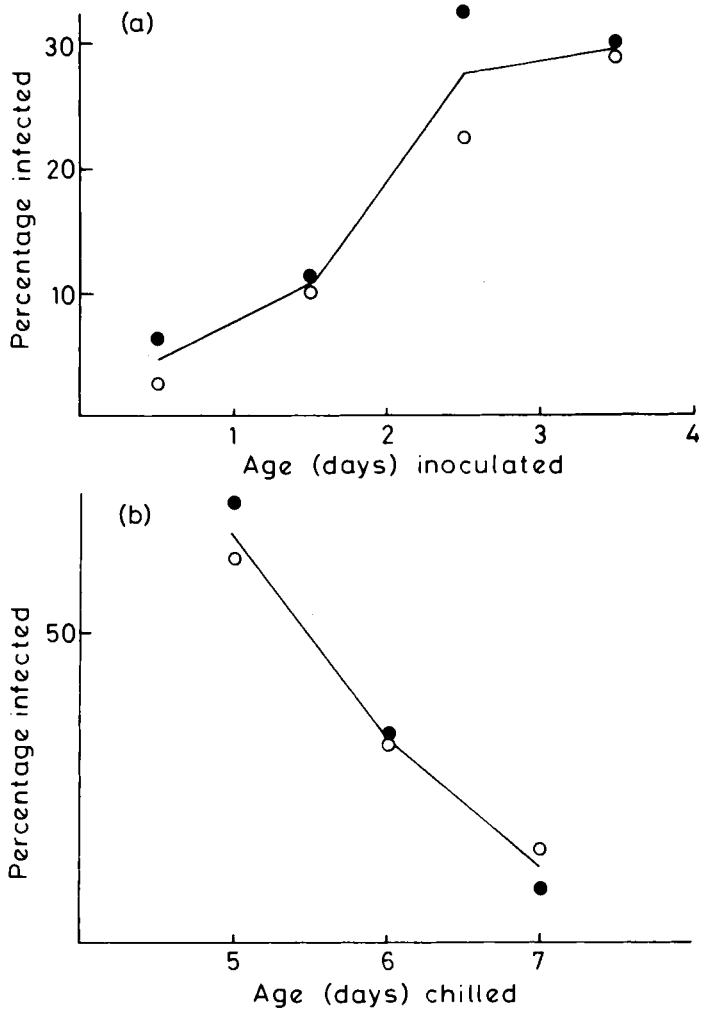

Figure 14 (a) Percentages of groups of 50 larvae that developed chalk-brood after each larva was inoculated with 10^4 spores at the age indicated and cooled temporarily to about 25°C when 5 days old. (b) Percentages of groups, of 130 or more larvae, that developed chalk-brood after each larva was inoculated with 10^4 spores when 3–4 days old and cooled temporarily to about 25°C at the age indicated; ● Colony 1; ○ Colony 2. (After Bailey, 1967c.)

D. *Spread*

Heath (1982a, b) makes it evident that *A. apis* is widespread throughout Britain and states that in south-west England "it has been possible to detect *A. apis* in any colony of bees . . . if the search is diligent enough". Clearly then, spread of the disease within colonies is almost entirely suppressed spontaneously.

Each larva that is killed by chalk-brood and produces cysts forms about 10^8–10^9 spores. Most of these are ejected from the colony by the house-cleaning bees that remove dead larvae from their cells, but many will inevitably find their way to healthy larvae via mechanical contamination on nurse bees or become lodged in food stores, and especially in brood comb (Koenig *et al.*, 1986), where they stay infective for many years.

The spread of chalk-brood within colonies is probably much suppressed by the normal temperature of the brood-nest or the absence of possible predisposing factors (Section C.); but some further limitation would seem to be imposed by the need for at least two spores of different strains to germinate and mate within a larva to form fruiting bodies. This limitation is indicated by the many chalky-white larvae and often very few larvae with fruiting bodies in typical outbreaks of disease. Indeed, combs have been found with all the dead larvae apparently infected with one strain of the fungus (Maurizio, 1934). This implies infection stemming from a single larva that had been infected with only one strain, and that larvae can become infected with mycelium as well as spores, which seems improbable. A more likely answer may lie in the observations of Christensen and Gilliam (1983) who isolated both strains from larvae that nevertheless appeared chalky-white. They suggested that unknown conditions within the gut sometimes favour the multiplication of one strain over the other. Consequently, larval remains may become overgrown and depleted by one strain before the other has time to mate and form cysts.

E. *Occurrence*

Chalk-brood occurs widely in the temperate regions of the Northern Hemisphere. It has long been known in Europe, Scandinavia and Russia (Betts, 1932) and also in New Zealand (Seal, 1957). Not until about 1970 was it officially recognized in the USA and Canada, where it was found especially in the mid-western and western regions. Since then it has been detected in Argentina, Japan, the Philippines, central America and Mexico (Heath, 1985). It was said to be "the most widespread infectious disease" in Mexico (Wilson *et al.*, 1984); and to have been "most serious" in Norway in 1977 and "alarmingly on the increase" in Britain in 1932 (Heath, 1985); but there have been no data or subsequent events to support these affirmations.

There has been much discussion about its origins and spread between countries, but the sequence of its detection may be as a result of an increasing awareness and interest by investigators. There are suspicions that it was in N. America during the 1920s but some investigators are convinced that the incidence of chalk-brood has increased in recent times (Heath, 1985). There are the usual suggestions of more susceptible strains of bees but a more likely change, if any has occurred, is that different strains of the fungus have become established, perhaps in solitary bees which have been cultivated and distributed on a large scale in the USA for many years, particularly for the pollination of alfalfa. They have suffered greatly from their own species of *Ascosphaera*, probably as a result of the increasingly industrial style of their management. *Ascosphaera apis* has been isolated from some of them (Section D.) and there may be strains of increased virulence for honey bees among them. Newly emerging healthy adult leaf-cutter bees have to chew their way through any larvae killed by the fungus, or through contaminated nest material in the tunnel ahead of them; and each can then carry between 10^4 and 10^7 spores on their bodies (Vandenberg *et al.*, 1980). Some of these spores may find their way to honey bees foraging on alfalfa.

Although Heath (1985) found no cultural differences of any significance between American and British strains of *A. apis*, Gliński and Chmielewski (1982) reported up to 20-fold differences between the virulence of some of the 40 strains they tested on young honey bee larvae.

F. *Cultivation of* Ascosphaera apis

Ascosphaera apis can be cultivated on potato-dextrose agar + 0.4% yeast extract, or on malt agar (0.5 to 2% malt). Strong vegetative growth with aerial hyphae and abundant fruiting occurs on the potato medium, but the malt agar has been found better for microscopic work as aerial hyphae are then virtually absent. Others have found that *A. apis* needs complex nitrogen sources and grows best in media with 0.1% asparagine and 0.5% yeast extract with the pH near neutral but below 7.2. The optimum temperature for growth is about 30°C.

Very small inocula of spores do not germinate well on the media mentioned above but will do so readily beneath the surface of a semi-solid agar of the yeast-glucose-phosphate medium suitable for cultivating *Melissococcus pluton* (Chapter 4, II.F.; Bailey, 1967c). This probably depends upon their need for a fairly high concentration of CO_2, in addition to moisture, to become "activated", i.e. enter the phase of swelling and germinating (Heath and Gaze, 1987). Most become activated within 10 minutes when moistened and exposed to 10% CO_2; and if some metabolic process that produces CO_2 begins when spores take up water and possibly nutrients, more CO_2 will accumulate in

spores beneath the surface of the agar than in those on its surface. Once the spores germinate the mycelia grow to the surface where spore cysts form when two strains meet. Mycelium does not grow below about 15 mm in the medium, which has a very stable oxidation potential (Bailey and Gibbs, 1962), unless it is incubated below 35°C, which enables enough oxygen to penetrate deeper. This seems analogous to events in nature, the mycelium being unable to grow enough in the almost anaerobic gut contents to reach the tissues, unless the metabolic rate of larvae is decreased by chilling or perhaps in other ways (Section C.).

It remains to be seen whether germination of small inocula of spores on the surface of agar is facilitated by an atmosphere of 5 or 10% CO_2 in air, as for *Bacillus larvae* (Chapter 4, F.), but this would seem very likely.

II. STONE-BROOD

A. *Symptoms*

Larvae with stone-brood may be sealed or unsealed. At first they are white and fluffy and later turn a pale brownish or greenish-yellow and become very hard. Most die after they have been capped in their cells prior to pupation.

B. *Cause*

Stone-brood is caused by *Aspergillus flavus* or, less often, by *Aspergillus fumigatus*. Mature growth of *A. flavus* has a yellow-green appearance and that of *A. fumigatus* appears grey-green. Both look similar under the microscope (Fig. 15) and both fungi are common, occurring in soil and cereal products. They infect and kill other insects and sometimes cause respiratory diseases in animals, particularly man and birds.

C. *Multiplication and Spread*

Spores can germinate on the cuticle of larvae, and the mycelium will then sometimes penetrate the sub-cuticular tissue and produce local aerial hyphae and conidiophores; but it seems that infection is usually via the gut (Burnside, 1930). The internal tissues are quickly overgrown with mycelium which eventually breaks through the cuticle near the anterior end of the body and then grows closely over the cuticle to form a false skin within 2 or 3 days. Conidiophores begin to form at the same time, wherever the mycelium is exposed to the air.

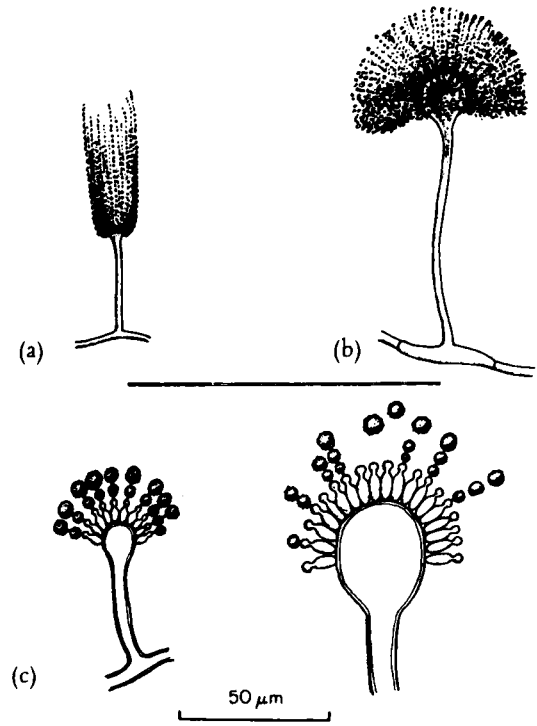

Figure 15 (a) *Aspergillus flavus*; (b) *Aspergillus fumigatus*; (c) *A. flavus*; detail of conidiophore. (After Dade, 1949.)

The fungi can multiply in adult bees and undergo a development in them similar to that which occurs in larvae (Burnside, 1930). However, the amount of mycelial growth in the gut of infected individuals, which become flightless and sluggish and crawl away from the cluster of bee colonies, seems inadequate to account for these early symptoms. These are probably caused by toxins liberated by the mycelium. An ether-soluble toxin occurs in cultures of *Aspergillus flavus*, particularly in cultures that have just reached maturity, which is indicated by the deep yellow-green colour of most of the conidia. The extracted toxin is unstable, losing most of its potency within 15 days.

Stone-brood is usually transient, although very large inocula can kill colonies (Burnside, 1930). The death of a few naturally infected colonies has been observed (Dreher, 1953). In these the mycelia had grown into the brood-comb cell walls, probably making it impossible for the adult bees to clean and disinfect cells.

Passage of *Aspergillus flavus* in an insect host raises its virulence (Madelin,

1960), so once established in a colony more virulent strains of the fungus might become selected.

D. *Occurrence*

Stone-brood is well known in Europe and North America. Infection of larvae by *A. flavus* has been reported from Venezuela (Stejskal, 1958). It is rare in Britain, however, in spite of the common occurrence of the fungi and of the damp climate, which is usually believed to encourage fungal infections. The fungi probably do not usually grow in bees and may do so only when bees are enfeebled by other factors.

III. OTHER FUNGI AND YEASTS

A micro-organism of a primitive type appearing to be transitional between yeasts and fungi has been found in the melanized (blackened) patches of epithelial tissue that surround nurse- and egg-cells of ovaries and the poison sac and rectum in queens: a disease originally called "eischwarzsucht" and renamed "melanosis" (Fyg, 1934). The organism is easy to culture *in vitro*, and adult workers and drones have been infected successfully with cultures injected into the thorax (Fyg, 1936). Bees that ingested the fungus did not become infected. Infected queens soon stop laying and are superseded by a new queen. One unmated queen was found by Fyg to have infected ovaries. The ease with which the organism is cultivated and its power to infect only after injection, suggest that it is a saprophyte which occasionally invades the bodies of bees via wounds.

Similar fungi and symptoms have been described in honey bees and in a solitary bee, *Andrea fulva*, by Örösi-Pal (1936, 1938b). However, Skou and Holm (1980) who investigated queens that were failing to lay many eggs, found many with melanosis but failed to associate the disease with any pathogen. They isolated many different micro-organisms, mostly yeasts, some resembling those described by Fyg, from the reproductive organs of the queens, and found the yeast *Saccharomycopsis lipolytica* the most common; but it was not pathogenic in experiments.

Other yeasts have been isolated on malt-agar from the intestines of sick bees and many bees died in colonies that fed on the yeasts supplied in syrup. Control colonies were unaffected. The pathogenic yeasts seemed to be *Torulopsis* spp., closely similar to *Torulopsis candida* (Giordani, 1952). Many

other fungi and yeasts that occur naturally in the gut of adult bees have been listed by Vecchi (1959) and by Skou and Holm (1980), but none seems to be pathogenic.

6
MICROSPORA AND PROTOZOA

The Microspora are a group of unique spore-forming organisms, many species of which are parasites of insects (Bulla and Cheng, 1976). One of the best known of these is *Nosema bombycis* which causes "pebrine" in the silkworm, *Bombyx mori* (Chapter 1). *Nosema apis* of the honey bee is another well-known species.

Until recently the Microspora were classified as Protozoa. These are microscopic, unicellular or acellular forms of animal life and comprise a great diversity of types. Many are parasitic or symbiotic in all forms of animals including the honey bee. Well-known symbiotes include those that dwell in the gut of termites and ferment cellulose, enabling the insects to subsist on a diet of wood. A related class of such organisms, but of unknown significance, live in the gut of honey bees.

1. *NOSEMA APIS*

Nosema apis develops exclusively within the cells of the epithelium of the mid-gut of adult bees (Fig. 41a). Many research workers have looked for its spores in tissues other than the mid-gut, but have usually found nothing. Microsporidian-like spores have been seen in other tissues, such as ovaries, fat-body and even hypopharyngeal glands of adult bees (Steche, 1960), and have been identified as those of *N. apis*, but only by their size which is an unreliable guide (Kramer, 1960a). Masses of particles that look like spores of *N. apis* and occur occasionally in honey have proved to be starch grains from maize or pollen (Sturtevant, 1919); and some fungal spores, e.g. of the common mushroom, are very like those of *N. apis*. Microsporidian-like spores have also been found in the blood, rectal glands and large flight muscles in the thorax as well as egg and nurse cells and other ovarian tissues and the mid-gut epithelium (Örösi-Pal, 1938b). These spores were seen in histological sections and were very sparse; no infection experiments could be made to

establish their identity. There may be microsporidia of other insects that sometimes infect honey bees. The species found attacking brood and killing pupae, but not affecting adult bees, in South Africa (Buys, 1972), and another with cylindrically shaped spores, found in adult bees in the USA (Clark, 1980), could be of this kind.

The host specificity of *N. apis* has been called into question. Fantham and Porter (1913) believed they infected several species of bumble-bees, wasps, moths and flies by feeding them spores; Showers *et al.* (1967) state that they similarly infected colonies of *Bombus fervidus*. Mescheryakov and Grobov (1983) report that they successfully infected cultures of mammalian tissues with *N. apis*, observing spores within 10% of the cells after 10 days. However, Kramer (1964) in well-controlled tests found that spores that were infective for honey bees did not infect muscoid flies, including species that Fantham and Porter said they infected. Whatever the issue, there is no evidence that infections of hosts other than honey bees are necessary for perpetuating infections in honey bee colonies (Section C.).

A. *Symptoms and Diagnosis*

Infected bees show no outward signs of disease. Even the mid-gut shows little evidence of damage when infected. The nuclei of infected cells seem normal, although finer details such as the striated border of infected ventricular cells and the formation of peritrophic membranes seem abnormal in severely infected bees (Hertig, 1923), and cytoplasmic granules of calcium phosphate, which are numerous in healthy ventricular cells, disappear in infected cells. However, infected bees live only half as long as non-infected individuals in colonies in spring or summer (Maurizio, 1946); the lives of infected caged bees are shortened between 10 and 40% (Bailey, 1958; Beutler and Opfinger, 1949); and infected bees do not fully develop their hypopharyngeal glands (Lotmar, 1936; Wang and Moeller, 1969), which probably explains why about 15% of the eggs in severely infected colonies fail to produce mature larvae in early summer, compared with about 1% in healthy colonies (Hassanein, 1951). Furthermore, infected bees in winter have only about 6 mg of nitrogen in their fat-bodies, whereas healthy bees have between 14 and 23 mg (Lotmar, 1939) and have more amino acids in their haemolymph than infected bees (Wang and Moeller, 1970b). The dry weight of caged infected bees, minus their alimentary canals, decreases more rapidly than that of caged uninfected bees, while their rectal contents gain weight more rapidly, so the bees become dysenteric earlier than uninfected individuals. The dysentery is mainly due to the accumulation of water (Chapter 9, I.), since the total water content of infected bees is higher than usual (Lotmar, 1951).

Although *Nosema apis* appears likely to aggravate dysentery, there is no

evidence that it is a prime cause of dysentery in nature. A survey of over 100 naturally infected colonies, during a winter when dysentery was prevalent, showed that although it was clearly associated with the death of many of the colonies dysentery was not caused primarily by *N. apis* because it occurred whether or not colonies were severely infected. Moreover, *N. apis* was not the prime cause of death of colonies because it was about equally distributed among living and dead ones (Fig. 16) (Bailey, 1967d). This distribution of *N. apis* was atypical because of the unusual amount of dysentery: characteristically, after most winters, comparatively few colonies become severely infected (Fig. 17).

Infected bees begin duties normally undertaken by older bees, i.e. young infected bees soon cease to rear brood and to attend the queen and they turn to guard duties and foraging (Wang and Moeller, 1970a). This is the same effect on adults as that caused by sacbrood virus (Chapter 3, II.C.). The

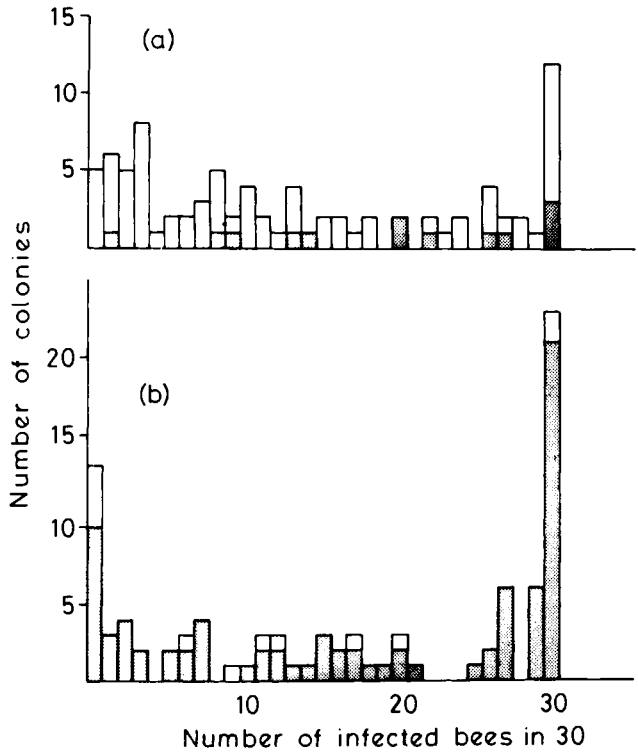

Figure 16 Distribution of infection with *Nosema apis* among (a) live and (b) dead colonies during March–April, 1963 at Rothamsted; and the distribution (stippled columns) of visible signs of dysentery among them. (From Bailey, 1967d.)

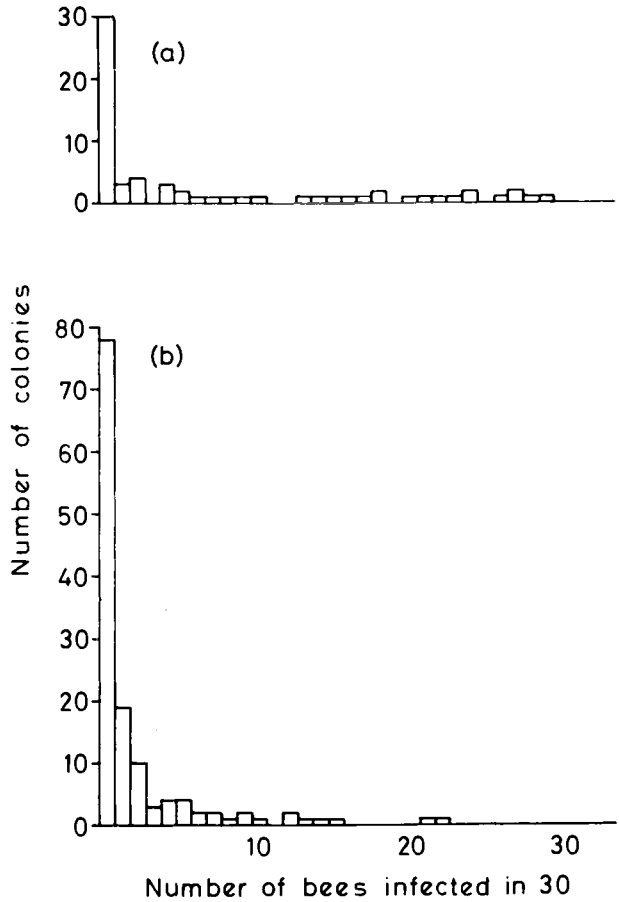

Figure 17 Distribution of infection with *Nosema apis* among live, seemingly healthy colonies after 2 average winters (a) 1964 and (b) 1965. (From Bailey, 1967d.)

effect of infection on the length of life of caged bees can be offset very largely by providing them with pollen additional to sucrose (Hirschfelder, 1964). Infected queens cease egg-laying and die within a few weeks of becoming infected (Fyg, 1948; L'Arrivée, 1965).

When colonies are artificially infected in spring or summer they recover in a few weeks, but when they are similarly infected in autumn they die in winter (Jamieson, 1955; Morgenthaler, 1941; White, 1919), because there is insufficient time for infection to decrease spontaneously (Section I.C.) before the bees cluster for the winter. Death of colonies, or serious damage to them, is rarely caused by natural infection; but significant negative correlations have

been reported between honey yields and the degree of infection (Fries *et al.*, 1984; Hammer and Karmo, 1947; Harder and Kundert, 1951; Poltev, 1953). Furthermore, significant increases in honey yields have been obtained by treating colonies with an antibiotic that is specific for *N. apis* (Chapter 10, IV.2.). In winter, infected colonies lose about 1500 more bees than normal colonies of the same size, regardless of their absolute size, i.e. the smaller the colony the relatively greater the loss of bees from infection (Jeffree, 1955; Jeffree and Allen, 1956). This suggests that small colonies are usually more severely infected than large ones and it might be supposed they are small because of the infection. However, undetermined factors additional to *N. apis*, but associated with it (because they are also associated with dysentery), are sometimes much more important inhibitors of the growth of colonies in spring (Bailey, 1967d).

Many of the pathological effects mentioned above may be due, in part, to one or more of the viruses that usually multiply only in bees already infected with *N. apis* (Chapter 3, V.).

Since there are no clear symptoms, the diagnosis of infection depends upon microscopic examination, for the presence of characteristic spores, either of extracts in water of bees, or of their faecal matter. This can be collected on horizontal glass plates mounted near the hive entrance (Wilson and Ellis, 1966), and it can be collected from queens without harm by holding them over glass slides (L'Arrivée and Hrystak, 1964).

B. *Multiplication*

The spores are ingested by the bee and are passed quickly into the mid-gut by the proventriculus. As soon as they enter the mid-gut they each extrude their hollow polar filament and inject the germ through it into an epithelial cell (Kramer, 1960b; Morgenthaler, 1963) (Figs 18, 41d).

The parasite develops and multiplies in the cytoplasm of the host cell, and in bees kept at 30°C the spores form after about 5 days. These are cast into the gut and pass to the rectum, often still inside the host cell (Fig. 41a) when, as in normal bees, this is sloughed off. All the cells of the mid-gut are eventually parasitized possibly by reinfection from newly-produced spores that have been cast off into the gut cavity, or by invasion of vegetative forms from adjacent cells as described for *Nosema bombycis* in the silkworm (Isihara, 1969). About 30–50 million spores are in the gut of a bee when infection is fully developed.

N. apis does not infect honey bee larvae (Hassanein, 1951). Newly-emerged bees are always free of infection, but they are as susceptible as older bees (Bailey, 1955a).

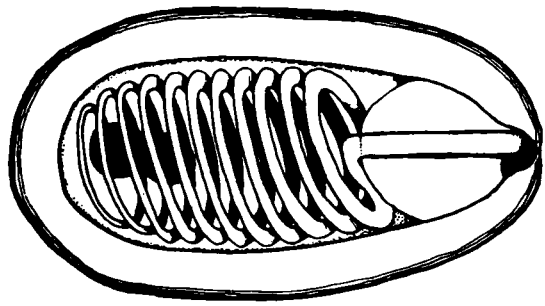

Figure 18 Internal anatomy of a spore of *Nosema apis*; diagram based on electron micrographs of ultrathin sections, showing the polar filament coiled round the twin nuclei of the sporoplasm. (After Huger, 1960.)

C. *Spread*

Spores were once believed to be disseminated by the wind, a variety of insects, and the flowers and water at foraging places visited by bees (Fantham and Porter, 1912, 1913; White, 1919). However, early work showed that infection declined spontaneously during the summer in North America and Europe after a spring peak (Bullamore and Malden, 1912; Morgenthaler, 1939; White, 1919). Suggestions were made that, although spores were being spread, high summer temperatures inhibited their multiplication in bees (Schulz-Langner, 1958). However, temperatures have to be raised to more than 35°C for many days to inhibit multiplication of the parasite in bees in the laboratory (Lotmar, 1943) and the temperature in the centre of the brood-nest rarely exceeds this (Ribbands, 1953) even on hot days. Higher temperatures outside the brood-nest could affect only a fraction of the adult bees for brief periods and cannot account for the spontaneous decline of infection in cool climates, such as in Britain. Here, experiments showed that the parasite multiplied as much as ever in marked infected individual bees that were placed in infected colonies, and then sampled at intervals during the period when the percentage of unmarked infected bees was declining rapidly and spontaneously (Bailey, 1959a). Therefore, the only explanation for the spontaneous decline of infection in summer is that infection does not spread from infected bees to the next generations of individuals.

Spores are spread in the faecal matter of adult bees and are ingested by young individuals when they clean contaminated combs. Bees are more likely to defaecate within the cluster during late winter, after their long confinement, than at other times, because the weight of their rectal contents then increases rapidly (Nitschmann, 1957). The sharp increase in weight is attributed to a

heightened metabolism at the start of brood-rearing whilst bees are still mainly confined to the cluster.

When bees are able to fly freely in summer and defaecate away from the colony the combs become cleaner and the chances of a bee contacting spores decrease. The infected bees die without transmitting their infection, which accordingly decreases. For the same reason spores of *N. apis* rarely occur in the honey or pollen collected by the bees from natural sources.

Queens do not clean combs so they rarely become infected under natural conditions. Drones become infected, but significantly fewer than workers although they are equally susceptible: they probably acquire infection by chance when they are fed by workers also engaged in cleaning combs (Bailey, 1972).

Colonies transferred carefully to non-contaminated combs in early summer lose their infection at about the same rate as untreated control colonies (Bailey, 1955b, c), but by the following spring the treated colonies have less, often no, infection, whereas the usual increase occurs in untreated colonies (Fig. 19 and Table VIII). Furthermore, combs taken from infected colonies in spring and placed in uninfected colonies in late summer, cause a resurgence of infection (Fig. 19). This dies away at about the same rate as in early summer, but has insufficient time to decrease as much as usual and so reappears in spring even more severely. These observations indicate that some infection persists on combs throughout the summer sufficiently to infect the winter cluster, and tests have shown that spores can survive dormancy in faecal deposits for at least a year.

Infection can remain endemic within a colony, but it is evident that recontamination of the comb by bees virtually ceases in summer. However, defaecation by bees can occur in the colony in summer when bees are confined by unusually inclement weather (Borchert, 1948), and there is a statistically significant positive correlation between cool, dull, rainy summers and the infection in colonies the next spring (Lotmar, 1943); although this may be partially because colonies grow slowly in rainy years and clean their combs less thoroughly than usual. Experiments showed that infection in spring was significantly less in colonies in which contaminated combs were put in the centre of their brood-nests during the previous summer, than in colonies in which contaminated combs were put on the periphery of the brood nests (Bailey, 1955b). This is because combs in the centre of the brood-nests are used more, and are therefore cleaned more, than those at the periphery. Colonies do not grow when they lose their queens and they stay more severely infected than usual (Table X; Chapter 10, IV.A.3.).

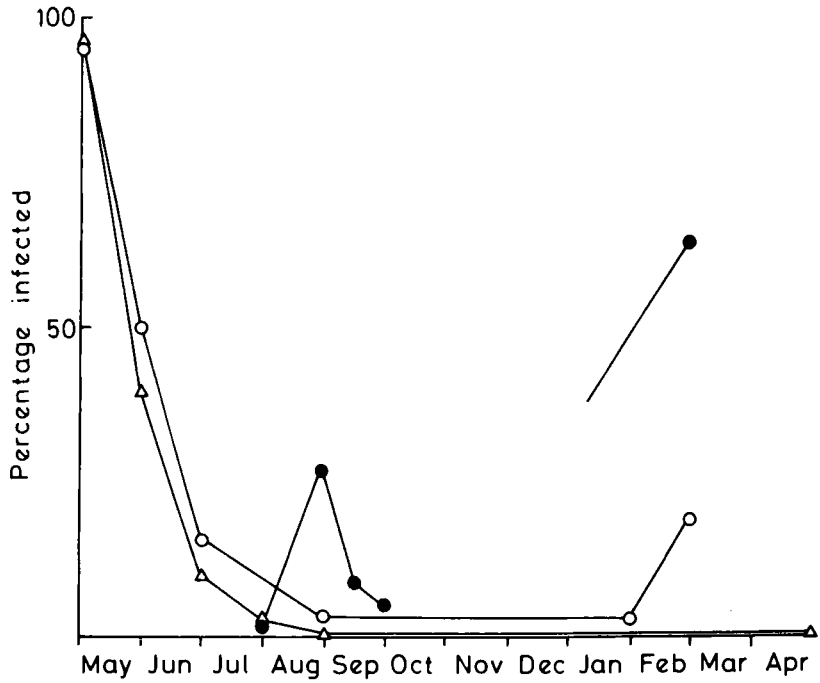

Figure 19 Average percentage of bees infected with *Nosema apis* in: 3 naturally infected untreated undisturbed colonies (○ — ○); 1 naturally infected colony transferred entirely to uncontaminated comb in June (△ — △); and 2 healthy colonies into which several combs, removed from severely infected colonies in spring, were placed in July (● — ●). Each point represents the average number of infected individuals in samples of 100 bees from each colony. (Partly from Bailey, 1955b.)

D. *Occurrence*

Nosema apis occurs in *Apis mellifera* throughout the world but has not been found in some parts of Africa, the Middle East, or the Malay Archipelago (Bradbear, 1988). Considerable differences have been reported between its incidences in different countries but the amounts found probably depend greatly on the scale and timing of the investigations. The true incidences are probably considerably greater than the values that have been reported. These range from less than 2% of colonies in Italy (Giavarini, 1956) to more than 60% in the Black Forest regions of Germany (Kaeser, 1954). However, most colonies are usually only slightly infected (Fig. 17). A survey in the USA showed that most queens in small colonies ("package bees") sent from the south to the northern states in spring were infected (Farrar, 1947); although

later, Furgala (1962) found very few, and Jay (1966) found between 4 and 20% only of queens were infected in similar packages, possibly because dealers had begun to take measures against infection (Chapter 10, IV.A.).

II. *MALPIGHAMOEBA MELLIFICAE*

This protozoan, also known as *Vahlkampfia mellificae* (Steinhaus, 1949) of the order Sarcodina, infects the lumen of the Malpighian tubules of adult bees (Prell, 1926), where it develops first as an amoeba-like individual which ultimately encysts (Fig. 41b, c).

A. *Symptoms and Diagnosis*

The epithelium of infected Malpighian tubules atrophies (Liu, 1985), but no other effects of infection have been found. Diagnosis depends upon the detection of cysts by microscopy, as for spores of *Nosema apis* (Section I.A.).

The effect of infection on colonies is uncertain; it is probably harmful but there are no known symptoms. The colonies used for the experiments described in Fig. 20 appeared normal at the height of infection. There have been reports of serious and even fatal infections, although the circumstances are not clear (Jordan, 1937).

B. *Multiplication*

Cysts ingested by the adult bee presumably germinate within the intestine, possibly at the posterior end of the ventriculus where solid food particles accumulate. The infective amoebae then probably enter directly into the Malpighian tubules, which discharge into the posterior end of the ventriculus, and apply themselves to the tubule epithelium. When it excysts the amoeba has a flagellated form that makes its way into the tubule where it changes into the trophic amoeba (Schulz-Langner, 1958). According to Fyg (1932), cysts form in bees kept at 30°C between 22 and 24 days after bees become infected, and then pass into the rectum to be discharged with the faeces.

C. *Spread*

In temperate climates there is a sharp peak of infection about May in the Northern hemisphere, followed by an abrupt decline, with infection becoming almost undetectable after midsummer (Hassanein, 1952; Poltev, 1953; Prell,

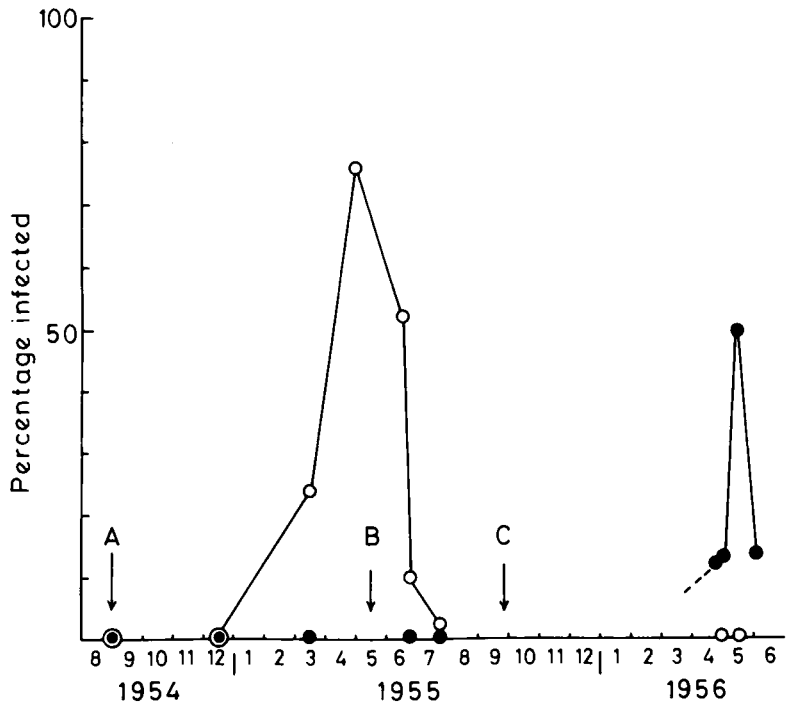

Figure 20 Average percentage of bees infected with *Malpighamoeba mellificae* in 2 bee colonies. A. One uninfected colony was divided into two; one of these (1, o — o) was given combs removed from colonies found infected with *M. mellificae* in April; the other half (2, • — •) was given similar combs that had been disinfected with acetic acid (Chapter 10, IV.B.1.). B. Colony 1 transferred to uncontaminated combs. C. Colony 2 transferred to comb removed in May from Colony 1. Each point represents the number of infected individuals in a sample of 100 bees. (Partly from Bailey, 1955d.)

1926; Schulz-Langner, 1969) (Fig. 20). The reason is the same as for the spontaneous decline of infection by *Nosema apis*. When combs of an infected colony are replaced by non-contaminated ones in summer, the infection does not reappear the next year (Fig. 20). Cysts disappear in summer much more abruptly than spores of *Nosema apis*, probably because cysts then have insufficient time to form in the short-lived bees. Natural transmission of infection to the winter bees is almost certainly by the remains of faecal contamination deposited on combs during the preceding late winter and spring. The apparent absence of infection in autumn and winter (Fig. 20), after contaminated combs are introduced, is in marked contrast to the surge of infection by *Nosema apis* when this is introduced in the same manner

(Fig. 19). Possibly the development of cysts is more retarded than that of spores of *N. apis* in bees at low temperatures. This could also explain the very steep rise of infection with *M. mellificae* in early spring, when the temperature of the cluster rises from about 20°C to about 30°C (Simpson, 1950).

M. mellificae is associated with *N. apis* in bee colonies more frequently than can be accounted for by chance (Bailey, 1968). The two parasites are independent, since they often occur alone, either in individual bees or even in colonies, but they become associated because they are transmitted in the same way. *M. mellificae* forms only about 500 000 cysts per bee and these take about 3 weeks to develop, whereas *N. apis* forms up to 30 million spores per bee in about half the time. Therefore, *M. mellificae* spreads less easily than *N. apis*, usually only by severe dysentery. Accordingly, it is usually associated with the most severe infections by *N. apis* (Fig. 21) and with unusual mortality of colonies, but it is not a prime cause of such losses.

Fyg (1932) was unable to infect seven queens with *M. mellificae* by feeding them cysts in syrup, although the queens were successfully infected with *Nosema apis*, spores of which were fed at the same time; and worker bees were successfully infected with both organisms. Fyg suggested that queens are resistant to infection, as they may be to the pyloric flagellates (Section IV.), but at least one naturally infected queen has been found (Ingold, personal communication).

Örösi-Pal (1963) failed to detect cysts in over 500 queens that seemed to be sick or that were surplus to requirements, but 3 out of 10 queens that he tried to keep in cages in the laboratory during the winter became infected. The attendant workers in the cages were changed every week or so and, although they were not checked for infection, they sometimes defaecated "excessively" and so would have transmitted any infection they had to the queens. Queens become infected rarely in nature, probably because their chances of ingesting cysts are fewer even than those they have of ingesting spores of *N. apis* (Section I.C.).

D. Occurrence

Malpighamoeba mellificae is widely distributed in the northern temperate regions except Norway, Spain and Japan; and it has been found in southern parts of S. America and Africa and in New Zealand. It seems absent from most tropical and sub-tropical regions (Bradbear, 1988). However, its incidence usually seems very low, e.g. 2% of colonies in England and Wales (Anon., 1947–1980), 0.2% in Italy (Giavarini, 1956) and none in Scotland (Murray, 1952).

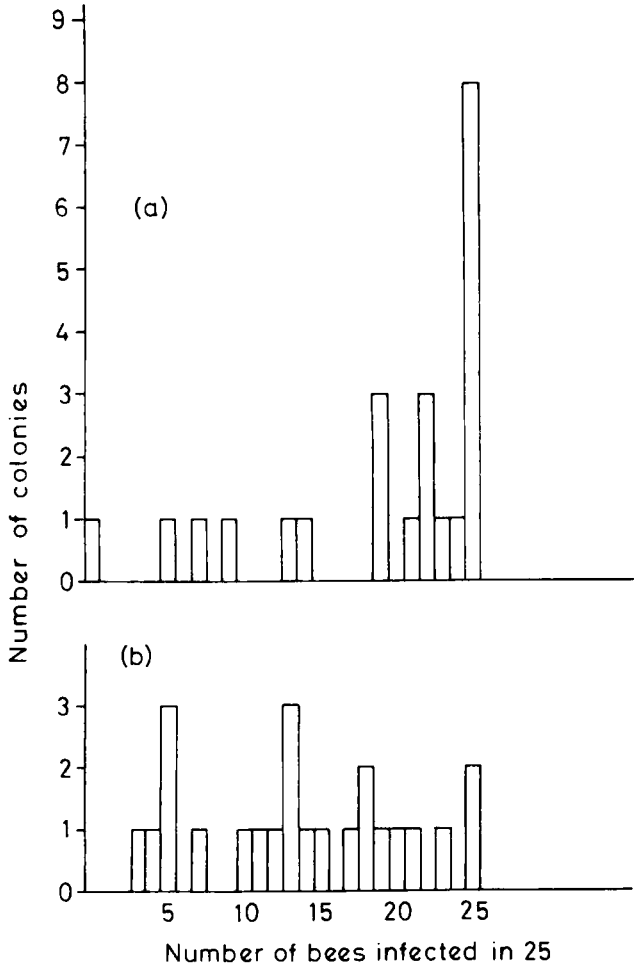

Figure 21 Distribution of infection with *Nosema apis* (a) and *Malpighamoeba mellificae* (b) among colonies infected with both parasites (compare with Fig. 17). (From Bailey, 1968.)

III. GREGARINES

These large protozoa (*Leidyana* and other spp.) live in the lumen of the midgut of adult bees attached to the epithelium (Fig. 41e) and may not be specific to honey bees (Wallace, 1966). Similar specimens have been found in Orthoptera and Lepidoptera. Those in bees may originate in nectar-gathering insects or in insects that sometimes infest honeycombs, such as cockroaches,

which live in weak colonies in tropical areas (Hitchcock, 1948; Stejskal, 1955). Stejskal (1973) identified four species of gregarines in bees in Venezuela. Gregarines encyst in bees and are passed out via the rectum, but it is not known whether the cysts then mature to form infective spores as they do to complete their life cycle in other insects.

There is little evidence of harm done to bees by gregarines. Infection found in spring in one colony disappeared during the summer (Hitchcock, 1948). Sick bees were collected in October from a few colonies in Venezuela, each bee having about 3000 gregarines in its ventriculus, and the following June sick bees were found once more. However, as Stejskal (1955) reported that fewer than 50% of the sick bees were infected with gregarines, the observed disease could not have been caused entirely by the organisms. Harry (1970) found that gregarines caused no damage to the gut of the desert locust and had no effect on the females, although they caused the weight of males to decrease.

Gregarines have been found in bees in Switzerland (Morgenthaler, 1926), Italy (Giavarini, 1937), Canada (Fantham *et al.*, 1941), USA (Hitchcock, 1948; Oertel, 1965) and South America (Stejskal, 1955), but their incidence is probably very low. Oertel reported less than 5% of bees infected in Louisiana.

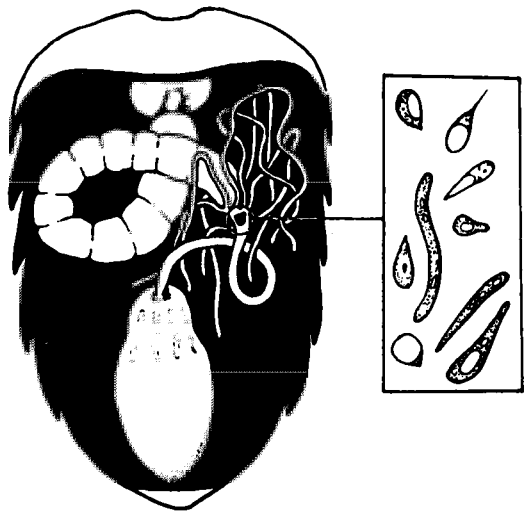

Figure 22 Flagellates in adult honey bees. The dark spot within the pyloris (ringed) is caused by the accumulation of flagellates illustrated on the right. (After Lotmar, 1946.)

IV. FLAGELLATES

A small area of the epithelium on the dorsal side of the pylorus of adult bees is frequently colonized by protozoa tentatively named *Leptomonas apis* (Lotmar, 1946), but re-identified as *Crithidia* spp. by Lom (1964). Infection causes a dark spot or crust, easily visible on the intestinal wall (Fig. 22), on which the flagellates are attached to form a "furry" coating. Rounded forms of these protozoa occur and may be cyst stages. Only 1 of 16 queens examined by Lotmar was infected, whereas most of the worker bees examined were infected. Flagellates apparently of the same type also occur in the rectum, either free in the lumen or attached to the epithelium (Fyg, 1954).

The flagellates do not occur in bees less than 6 days old. In newly-infected bees they move freely in the gut lumen, later they clump into rosettes and adhere to the intestinal wall where the dark crusts appear in bees more than 16 days old. They are scarce in winter bees; sometimes bees with the crusts have been found in winter, but with no flagellates (Giavarini, 1950).

There is no evidence that the flagellates are pathogenic. They have been found commonly in Europe and Scandinavia (Lotmar, 1946) and in Australia, where they were named *Crithidia mellificae* (Langridge and McGhee, 1967). They will multiply on artificial media (Fyg, 1954). They may not be specific to honey bees (Wallace, 1966).

7
PARASITIC MITES

There are many different kinds of mite (Acari) that parasitize a wide range of insects as well as other animals. They live and multiply on almost any surface, external and internal, of their hosts; and frequently become highly specific to a certain region, usually a particular groove, cranny or pit from which they are not easily dislodged. Some, for example, spend their lives in the tympanic organs of certain moths, using special sites within the organ for specific activities such as feeding, moulting, and breeding; amazingly, never infesting both ears of the same moth and so not incapacitating their host (Treat, 1958). Many species have taken to living within the tracheal system of insects, including locusts and bumble-bees. Examples such as these serve to show that the several kinds of similar mites that commonly infest honey bees are not so remarkable as they seemed when first discovered. More than 40 species of mites have so far been associated with honey bees (Eickwort, 1988). Six are known that parasitize *Apis mellifera* (Table VIII).

1. *ACARAPIS WOODI*

This mite (Fig. 23) first called *Tarsonemus woodi* (Rennie *et al.*, 1921) was later renamed *Acarapis woodi* (Hirst, 1921). It infests mainly the tracheae that lead from the first pair of thoracic spiracles of adult bees (Figs 24, 42), but mites have also been found in air sacs in the head and abdomen (Prell, 1927).

When congo red is injected into the haemolymph of infested bees, both adult and larval mites quickly turn red; so it appears they feed on the haemolymph of their host by piercing the tracheal walls with their mouth-parts (Örösi-Pal, 1934).

Table VIII Parasitic mites of honey bees (*Apis* spp.).[1]

Family	Species	Host species	Range
Tarsonemidae	*Acarapis woodi*	*cerana*, (*dorsata*,[2]) *mellifera*	Almost universal
	A. externus	*cerana*	*Japan*[3]
		mellifera	Almost universal
	A. dorsalis	*mellifera*	Universal
Varroidae	*Varroa jacobsoni*	*cerana*, *mellifera*	Almost universal
	V. underwoodi	*cerana*	Nepal
	Euvarroa sinhai	*florea*	Asia
Laelapidae	*Tropilaelaps clareae*	*cerana*, *dorsata*, *florea*, *laboriosa*, *mellifera*	Asia
	T. koenigerum	*dorsata*, *laboriosa*	Asia
Erythraeidae	*Leptus* spp.[4]	*mellifera*	Universal

1. After Eickwort (1988), Delfinado-Baker and Aggarwal (1987a,b) and Delfinado-Baker *et al.* (1985).
2. Requires confirmation (Delfinado-Baker, personal communication).
3. Prof. M. Sasaki (personal communication).
4. Infrequent larval parasites of adult bees.

A. *Symptoms and Diagnosis*

There are no outward signs of infestation by *Acarapis woodi*. It can be detected only by dissection to expose the main tracheae leading from the anterior thoracic spiracles. One of the simplest ways of doing this is described by the Ministry of Agriculture, Fisheries and Food (Anon, 1986a; Fig. 42a, b). Irregular dark stains develop in infested tracheae, the whole of which eventually blacken in infestations of long duration (Fig. 42b). Such deeply stained tracheae seem brittle compared with normal ones but, apart from these effects, little or no internal damage has been detected in infested bees.

The thoracic tracheae leading from the first thoracic spiracles are the main ducts for air, at least into the thorax, and they undoubtedly supply oxygen to the flight muscles. It has frequently been assumed that numerous mites in these ducts partially suffocate the bee and at least impair its ability to fly. However, this is not supported by direct observation. Bees severely infested with mites forage for pollen and nectar in an apparently normal way (Rennie *et al.*, 1921); and the same proportion of individuals with severely infested tracheae, some with both tracheae infested, occur among flying bees and those from the cluster of the same infested colonies (Bailey, 1958).

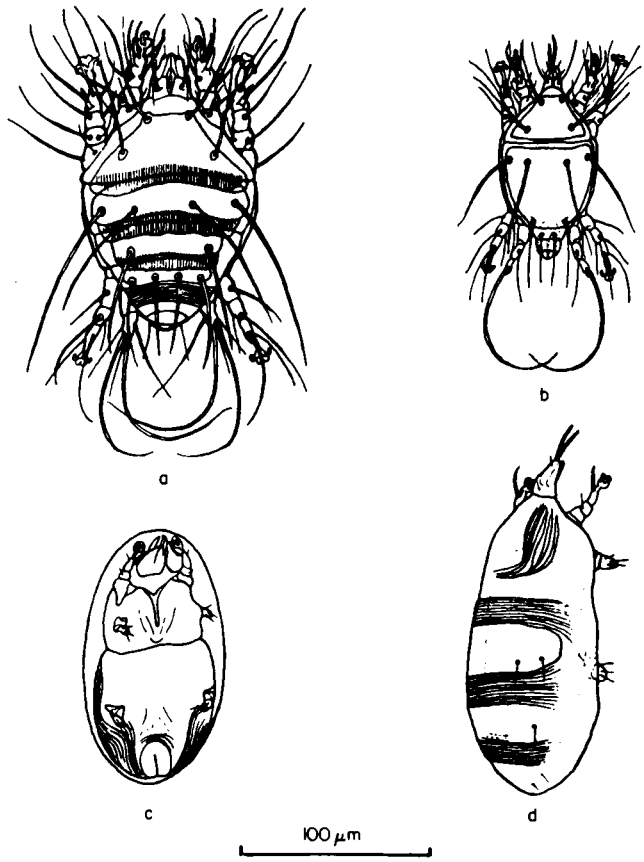

Figure 23 *Acarapis woodi*: (a) mature female, (b) mature male, (c) larva within its eggshell, (d) mature larva. Sizes vary, but males are always smaller than females. (After Hirst, 1921.)

Reports of severe damage of colonies by *A. woodi* abound in beekeeping journals and have become widely accepted, but no experiments were done to measure the effects of the mite until the late 1950s and early 1960s. It was shown then (Bailey, 1958, 1961) that overwintered infested bees die sooner than uninfested individuals and the difference became statistically significant from about March onwards (Fig. 25). This was done by marking every bee in moderately infested colonies during the autumn and then sampling the marked bees at intervals during the following winter and spring. As bees more than a few days old are virtually immune to infestation (Section C.; Fig. 28), the sharp fall in the percentage of infested individuals among the marked bees during the spring, showed that infestation was then shortening

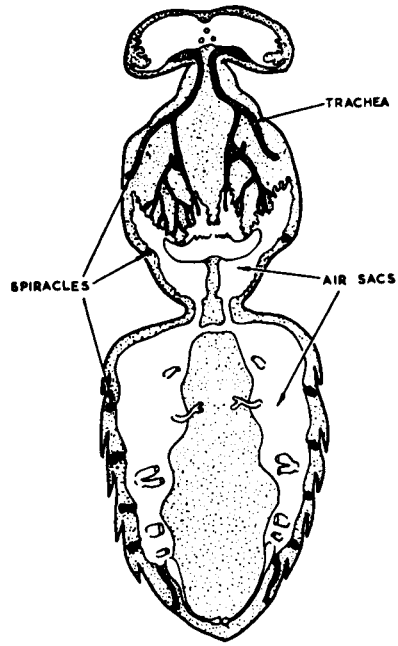

Figure 24 Tracheae and air-sacs of the adult bee. (After Snodgrass, 1956.)

their lives. Overwintering bees are all near the end of their lives by then, and are being replaced by new generations, so there were relatively few marked bees left to sample, and the effect of infestation on the colonies was not noticeable. When colonies are severely infested, most of their adult bees will die slightly earlier than usual, and this may not be sufficiently counter-balanced by the production of new bees. Thus, severely infested colonies dwindle more than usual; and colonies with more than about 30% of their individuals infested are more likely to die in spring than the rest. However, relatively few colonies become so severely infested in Britain (Fig. 26), where something less than 2% of colonies had measurably poorer performances that could be associated with infestation during the 1950s (Bailey, 1961). Most of this small proportion survived, and infestation in most of the survivors diminished during the active season to a low level. In more recent times the effects have been even less, as discussed in Section E. Field observations, including those of the lower honey yields (Eischen *et al.*, 1989) and rates of brood-rearing (Otis and Scott-Dupree, 1989) associated with severe infestations, provide circumstantial evidence of the effects of *A. woodi*; but many losses may be due, in part at least, to other factors that are independent of *A. woodi* but are aggravated by or associated with the same conditions that

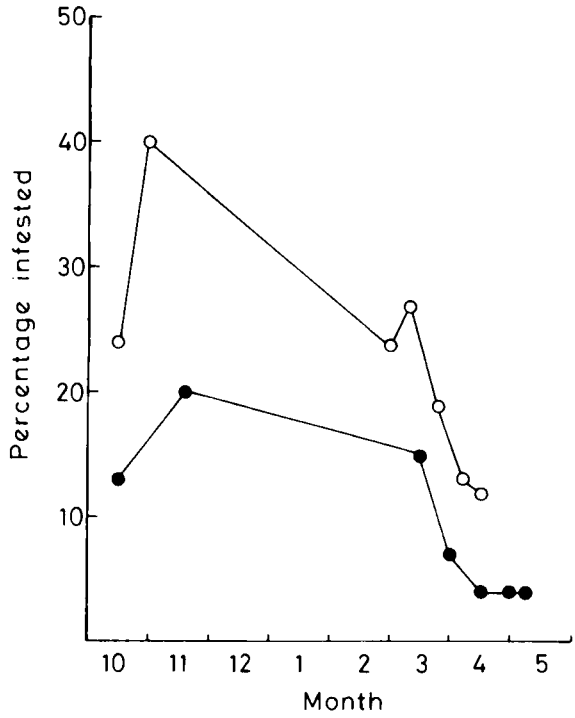

Figure 25 Percentage of marked individual bees infested with *Acarapis woodi* in 2 colonies, every bee of which was marked in October 1956 (● — ●) or October 1957 (○ — ○). Each point represents the number of infested individuals found in a sample of 100 marked bees. (From Bailey, 1958.)

allow mites to multiply and spread (Section E.). These factors, when unquantified, or, more likely, unknown, invalidate attempts to establish a cause and effect relationship between the performance of colonies and degrees of infestation that are not controlled experimentally. Clearly the death of colonies in winter in Britain is inevitable only when almost all their bees are infested, and even then other factors are almost certainly involved.

It was popularly supposed, and is still believed by many, that *A. woodi* causes the crawling and death of bees in summer, leading to severe dwindling of colonies (Chapter 9, V.). However, field experiments have shown that infested bees in summer die only a little sooner than uninfested individuals, and they appear normal until they die (Bailey and Lee, 1959). Fyg (1964) observed that infested queens can live "for many years". Some of the best evidence in this respect comes from Gary and Page (1989) who observed marked individuals in colonies in the USA that had about half their bees

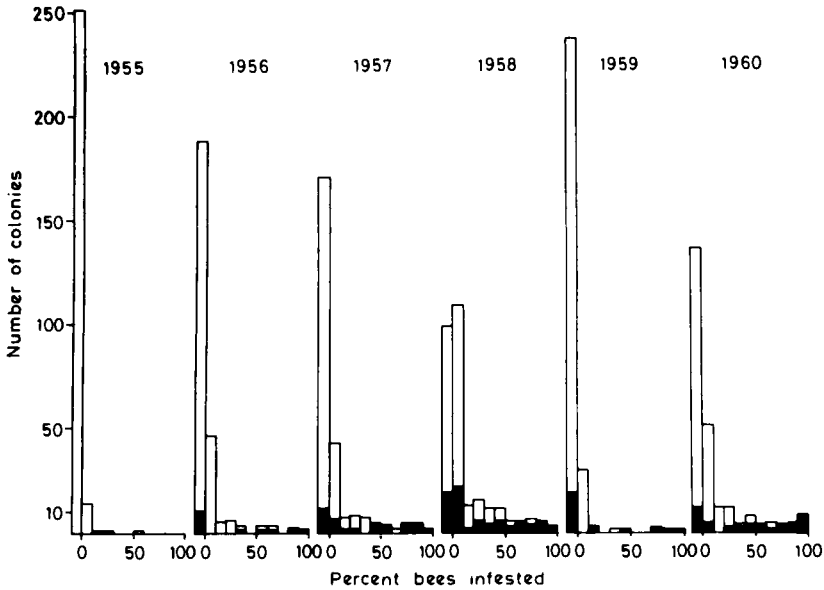

Figure 26 Natural infestations of untreated honey bee colonies with *Acarapis woodi* in autumn and mortality of colonies in winter at Rothamsted. Numbers of colonies that died during the winters of 1956–1959, inclusive, shown in black. Summers of 1955 and 1959 were the only seasons in the series during which bees accumulated honey surplus to their needs.

infested. They found no significant difference between infested and non-infested bees in the number of foraging trips they made, whether for pollen or nectar, and the time they took over them; and there was no difference between the nectar loads or the lengths of life of the bees. Gary and Page concluded: "There is a strong indication that tracheal mite infestations do not have a detectable economic effect on honey bee colonies when brood-rearing is active and honey production is under way".

The common occurrence of *A. woodi* makes it certain that many colonies suffering from any disorder will also be infested to some degree with mites. Some investigations of sick colonies found severely infested in summer with mites showed that, whereas both sick and apparently healthy bees from the colonies were infested about equally with *A. woodi*, the sick bees only were all infected with chronic paralysis virus (Chapter 3, I.A.; Bailey, 1969b). Similarly in northern India, where infestation of *Apis cerana* with mites resembling *A. woodi* is common, a "clustering disease", which leads to the death or severe dwindling of colonies in summer, was attributed to the mites. In fact, the mites were often very few or absent from the diseased bees, but

all of these were infected with *Apis* iridescent virus (Chapter 3, II.D.).

Various disorders, most notably paralysis, are frequently attributed to *A. woodi*, and this, more than anything, has led to the confusion of thought and terminology expressed by the commonly used term "Acarine or Isle of Wight disease" (Chapter 9, V.A.). Much the same conclusions—that damage attributed to *A. woodi* is frequently exaggerated or is often an inadequate or even mistaken diagnosis—have been reached after many observations in mainland Europe by Wille *et al.* (1987).

Infested bees have more bacterial infection, particularly in their haemolymph, than uninfested bees (Fehl, 1956), and this may sometimes cause disease. The bacteria presumably invade the haemolymph via wounds made by mites in the tracheae. On the whole the bacteria found in the haemolymph correspond with those in the tracheae, not those elsewhere in the bee. However, infestation with *A. woodi* does not increase the susceptibility of bees to disease when sprayed with suspensions of pathogenic bacteria or of viruses (Bailey, 1965a).

B. *Multiplication*

Female mites collect within the tracheae of worker bees within 24 h after the bees emerge from their cells and the ratios of male to female mites found within tracheae range from about 1:3 (Morgenthaler, 1931) to 3:1 (Otis *et al.*, 1988). Single female mites lay 5–7 eggs after 3 or 4 days, and after a further 3 or 4 days the eggs begin to hatch. The first males occur on the eleventh or twelfth day, the first females on the fourteenth or fifteenth day. Other observations have been that the eggs hatch 5 or 6 days after they are laid, and that female larvae become adult mites after a further 6 to 10 days (Örösi-Pal, 1935). Therefore, even when female mites mate and migrate immediately on becoming adult, the youngest worker bee that can possibly transmit mites will be 14 days old.

C. *Spread*

The proportion of infested bees in any colony often fluctuates widely during short periods (Fig. 27), which indicates an unstable dynamic equilibrium between the mortality of infested bees and the numbers of healthy bees becoming infested.

Only adult bees under about 9 days old are susceptible to infestation, and their susceptibility diminishes rapidly from the moment they are newly emerged from their brood-cells (Fig. 28), although the reason for this is obscure. Some believe it is because the hairs of bees stiffen with age and the

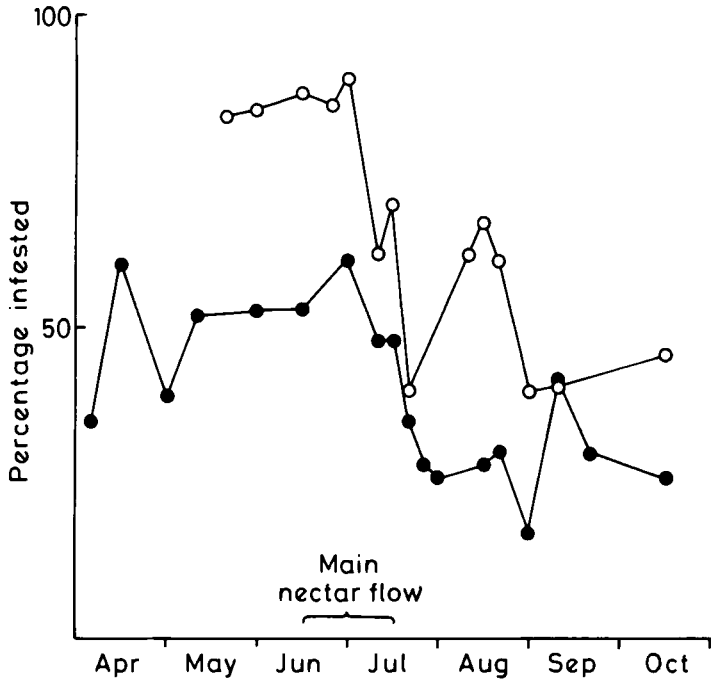

Figure 27 Percentage of individuals infested with *Acarapis woodi* in 2 individual bee colonies; each point represents number of infested individuals found in a sample of 100 bees (From Bailey, 1958.)

dense barrier of hairs at the entrance of the first thoracic spiracles thus becomes impassable (Sachs, 1952); mites can leave the spiracle when migrating, supposedly because the hair-barrier, like a valve, acts only one way. However, when newly emerged and older bees, each with one spiracle denuded of hairs, were introduced into cages of infested bees, some young ones but none of the old ones became infested, and the presence or absence of spiracular hairs had no effect (Lee, 1963). Therefore, old bees may not be physically immune to infestation but usually fail to attract mites. Queens are similarly resistant (Pettis *et al.*, 1989).

When a mite leaves a bee via the first thoracic spiracle it climbs a hair, usually on the thorax, and clings near its tip with one or both hind legs (Fig. 42d). It grasps with its forelegs a hair of another bee brushing past and descends to the surface of the new bee's body. Mites are then attracted to the region of the first thoracic spiracle by vibration of the wing roots nearby, and then to the spiracular openings by the puffs of air coming out of them, which are caused by the respiratory movements of the abdomen (Sachs, 1952).

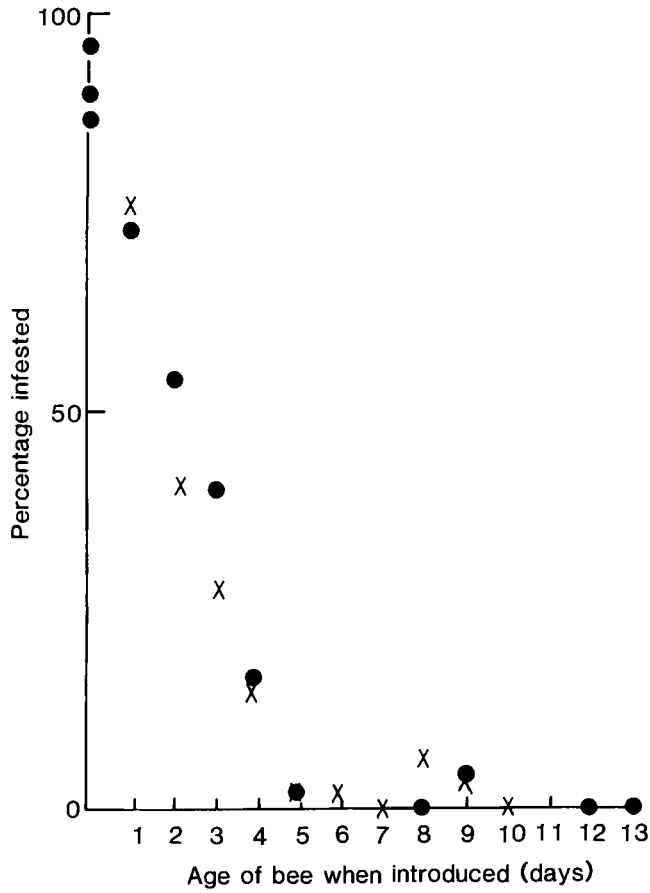

Figure 28 The percentages of bees that became infested with *Acarapis woodi* when introduced at different ages into severely infested colonies in Europe (•) (from Morgenthaler, 1930, 1931) and in Florida (×) (from Gary *et al.*, 1989.)

Mites migrate only in this fashion: they are unable to find a new host via honeycomb or flowers (Hirschfelder, 1952). They are much inhibited by a wire-gauze screen separating infested bees from young uninfested individuals, even though the bees are able to feed one another through the gauze (Morgenthaler, 1931; Pettis *et al.*, 1989).

Infestation increases in an endemically infested colony when relatively few young bees are available, because there are then many migrating mites for each available host. It also increases when the foraging activity of old bees becomes suppressed, because there is then more chance of contact between

old infested bees and young susceptible ones. These two situations occur together to a large extent; depressed brood-rearing coincides on the whole with times of decreased foraging activity, usually due to a failed nectar-flow, or when the colony density (the number of colonies per unit area) is too high for the available nectar.

Other beekeeping activities that suppress foraging when young bees are present also aggravate infestation, and include keeping colonies without queens for several weeks during the summer (Bailey and Lee, 1959).

When foraging increases during nectar-flows, infestation decreases (Figs 26, 27) because the old infested bees become further separated from the young susceptible individuals, and they eventually die with their mites in the field. The effect has been observed many times. Perepelova (1927) found that infestation declined in Russia during spring and summer, and that mites were hardly detectable by late summer. Average infestation in severely infested colonies taken from near Mexico to Nebraska declined by 80 or 90% in two successive seasons when nectar was being gathered (Cox *et al.*, 1988, 1989b; Moffett *et al.*, 1988); infestation declined from levels between 20 and 90% to between 3 and 34% in tropical areas of Mexico during nectar-flows (Lozano *et al.*, 1989); and infestations have been reported to fall from 90% to 5% during nectar-flows in Florida (Taber, 1987). Infestation in colonies taken to Ameland (Netherlands), where conditions were described as "favourable" for bees (Ruijter and Eijnde, 1987), became undetectable by the end of their second season without treatment with acaricides. Even in poor seasons in Britain the natural suppression of infestation remains strong and relatively few colonies become severely infested.

In winter, when there are very few or no young bees, the incidence of infestation remains fairly constant in the population of relatively insusceptible old bees, but it decreases abruptly at the end of winter when they die a little sooner than the uninfested individuals (Fig. 25).

D. *Occurrence*

1. Europe

A. woodi was detected in honey bees in Switzerland and Russia only about a year after it was originally discovered, in Scotland, by Rennie *et al.* (1921). It had probably been long established in mainland Europe, soon being found there in many widely separated regions when migratory beekeeping and trade in bees were very limited.

2. America

In the Americas, the mite was first detected in 1944–45 in Argentina and Uruguay (Lozano *et al.*, 1989); and then, between about 1970 and 1980, it was reported from Brazil, Colombia, Venezuela and Mexico, in that order. It is very widespread in Mexico, where Wilson and Nunamaker (1985) found it in 23 of 32 states, with the notable exception of the Yucatan region, where most honey is produced. Finally it was found in Texas in 1984 (Delfinado-Baker, 1984).

During 1980 to 1984 samples of adult bees from about 5000 apiaries in the USA and Canada were examined specifically for *A. woodi* and *Varroa jacobsoni* (Section III.) (Shimanuki and Knox, 1989). Neither mite was detected, which shows that infestation must, at most, have been very slight. This was shown again by another survey (Anon, 1984), when *A. woodi* was detected in fewer than 2% of 7000 samples, mainly from Texas and Louisiana, shortly after the mite had been first found there. However, remarkably high infestations with up to 100% of individuals infested per colony were under observation in some apiaries in New York State as early as 1985 (Otis *et al.*, 1988; Section E.2.).

Infestations soon found almost throughout the USA were attributed to the sale of bees and to migratory beekeeping; activities which are centred primarily in the southern states (Delfinado-Baker, 1985). The origin of the infestations is assumed to be Mexico, and the American strains of *A. woodi* are usually assumed to be the same as those in Europe.

3. Other regions

Outside Europe and the Americas, *A. woodi* has been identified in *Apis mellifera adansoni* in the Belgian Congo (Benoit, 1959), in Egypt, and in *Apis cerana* and *Apis dorsata* in the Indian sub-continent (Atwal, 1967; Delfinado-Baker *et al.*, 1989; Milne, 1957). It is usually supposed that the mite was taken to these lands in *A. mellifera* imported from Europe. If so, the seeming absence of *Acarapis externus* (Section II.) from *Apis cerana* in India and S.E. Asia needs an explanation in view of its common occurrence on *A. mellifera*.

A. woodi has not been detected in Australia or New Zealand, perhaps for reasons discussed in Section E. Nor does it seem to occur in Scandinavia, perhaps because infested bees cannot survive their prolonged winters long enough to infest the next generations of young bees.

E. *Regional Variations of Infestation*

The reason for the sparseness or absence of mites from Australia, New Zealand and, until comparatively recent times, America, may be because bees

were taken to these parts of the world by man and infested bees did not survive the long and arduous journeys. Secondly, and perhaps more significantly, bees would have survived only where they could flourish. Early settlers would not have kept them otherwise. These factors could have eradicated any infestation, as discussed in Section C. However, in recent times great changes have occurred in beekeeping and some of these have almost certainly substantially altered the susceptibility of bees to infestation.

1. England and Wales

The longest record of levels of infestation has been that of England and Wales (Anon, 1947–1980). Here, infestation has declined ever since records began (Fig. 29). The usual speculative reasons given for this are treatments, or climatic changes, or the natural selection of resistant strains of bee, or a combination of all three. Treatments have almost certainly been far too inefficient and spasmodic to have exerted such an inexorable effect; and any climatic change is unlikely to be important because *A. woodi* flourishes in a much wider variety of climates throughout the world. If infestation had been particularly damaging and its decline had been due to the natural selection of resistant bees, then the honey bee population and beekeeping would be expected to have made some discernible recovery. They have not. They too have continued to decline—simultaneously with and at the same rate as infestation (Fig. 29).

The striking fact about infestation by *A. woodi* in England and Wales, made evident in Fig. 29 and Table IX, is its highly significant association with the number of colonies per unit area, or colony density (Bailey and Perry, 1982). The data in Table IX show that the percentage of colonies in which infestations were detected in two groups of apiaries with unusually high colony densities, in two counties of England about 1960, were several times higher than in all the other apiaries, each of which contained correspondingly fewer colonies. The high infestations in the two groups of crowded apiaries were about the same as those that had prevailed some 30 years previously throughout Britain when colony densities, judging from Fig. 29, probably had been correspondingly high.

The declining infestation with *A. woodi* almost exactly resembles the decline of the incidence of paralysis (Chapter 3, I.E.), which is entirely independent of *A. woodi*; and these facts indicate a fundamental relationship between colony densities and the incidence of pathogens that are transmitted by close contact between individual bees. The specificity of this relationship is shown by the unchanging incidence of the various brood diseases during the same period, and by some increase of the non-contagiously transmitted pathogens of adult bees, *Nosema apis* and *Malpighamoeba mellificae* (Anon, 1947–1980).

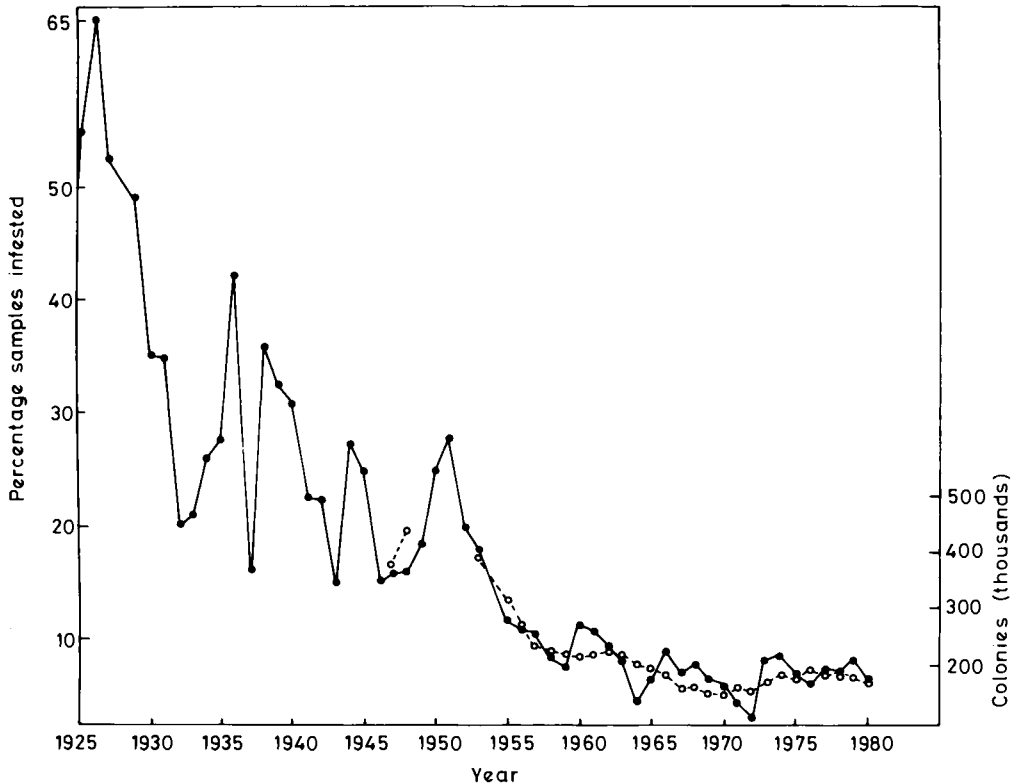

Figure 29 The percentage of samples of bees infested with *Acarapis woodi* (●) in England and Wales and the total number of bee colonies (○). (Data for 1925 to 1946 from Morison *et al.*, 1956; remaining data from Anon., 1947–1980, figure reproduced from Bailey and Perry, 1982.)

Table IX. Colony densities and infestation with *Acarapis woodi* (from Bailey and Perry, 1982).

Region	Locality	Period	Mean No. of colonies		Percent colonies infested
			Per year ($\times 10^{-3}$)	Per beekeeper or site	
Hertfordshire	All	1955–60	6.36 ± 0.72	5.1 ± 0.1	6.2 ± 1.8
	Rothamsted	1955–60	0.25 ± 0.01	14.0 ± 1.0	29.0 ± 7.4
	All	1960–67	4.05 ± 0.33	4.1 ± 0.3	3.4 ± 1.5
Oxfordshire	All	1955–65	3.62 ± 0.43	7.8 ± 0.8	7.5 ± 1.7
	11 commercial apiaries	1965	0.32	29.0 ± 1.0	40.0 ± 4.0
	All	1963–67	2.14 ± 0.26	4.8 ± 0.3	5.4 ± 3.5

The two latter parasites have multiplied more than usual probably because of less competition from the contagiously transmitted pathogens.

The possible reasons for declining numbers of colonies in England and Wales are discussed later (Chapter 9, V.A.); but, whatever the reason, its effect on the honey bee population has been contrary to what would be expected of increasing resistance to *A. woodi*.

2. North America

(a) Susceptibilities of bee strains It was long supposed that American bees would prove to be susceptible to the so-called "Isle of Wight disease", which was alleged to be caused by *A. woodi* (Chapter 9, V.), but tests with American strains of bee established in Britain (Bailey, 1965b, 1967a) showed that even the severest infestations did not cause any sign of such a disease; nor have there ever been any reports of it in the Americas.

Nevertheless, these trials did show that some strains of North American bees are susceptible to higher infestations on average than European bees when they are kept in Britain; although they indicated the possibility that the difference was due indirectly to a decreased foraging activity of American colonies in a less favourable environment than their native one, and not to a higher susceptibility of their individual bees. This possibility seems supported by the remarkably similar resistance to infestation of European and American bees with age (Fig. 28).

However, there have been indications that the distribution of infestations are biased towards higher levels in the USA than occur in Britain (Robinson *et al.*, 1986; Otis and Scott-Dupree, 1991; Fig. 30). Furgala *et al.* (1989) reported increases of infestations from an average of 1% of individual bees to about 50% in 17 months in 75 colonies in Minnesota, with only 11 of the colonies surviving two winters. The progress and distribution of these infestations among colonies in New York State and Minnesota would be unusual in Britain today, except in colonies kept for long in conditions below average for foraging. Even so, the relationship between the degree of infestation of a colony and the likelihood of its death in winter in N. America is much the same as in Britain (Otis and Scott-Dupree, 1989, 1991). Therefore there is nothing to suggest that there are any differences between the reactions of American and British bees to infestation.

Statistically significant differences between the susceptibilities to infestation of individuals of different strains of bees from widely separate regions of the USA have been detected by Gary and Page (1987). They compared the percentages of individuals that became infested, and the degree of their infestations, when newly emerged bees from different colonies were marked and then mixed, together with severely infested bees, in cages in the laboratory.

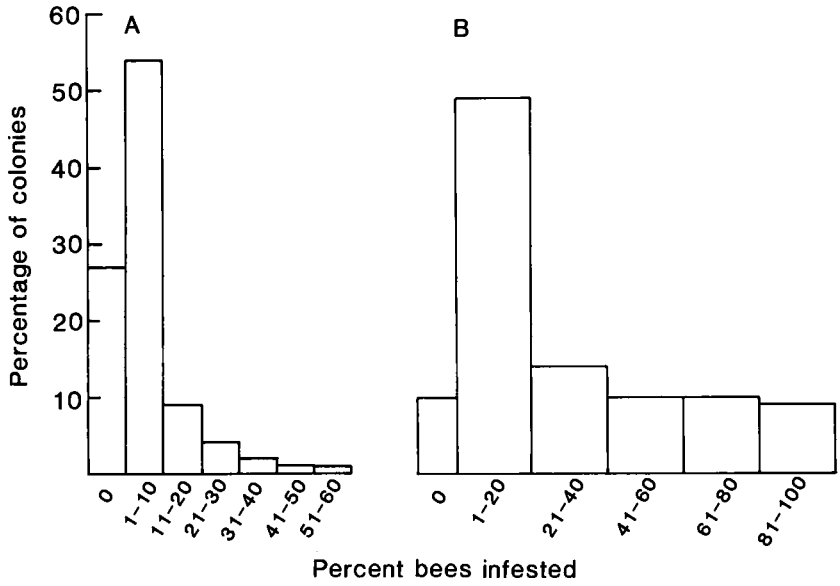

Figure 30 Average distributions of infestation by *Acarapis woodi* in several apiaries (A) in Florida (from Robinson *et al.*, 1986) and (B) in New York State (from Otis and Scott-Dupree, 1991.)

Individuals of a line selected for susceptibility for two generations were 2.4 times more likely to become infested than those selected for resistance (Page and Gary, 1990). Such tests would be expected easily to demonstrate substantial differences in susceptibility that some authorities consider are likely to arise between bees long exposed to infestation and those believed not to have been so exposed. However, when they used tests of this kind to compare bees from diverse sources in Britain with those of the United States, Gary *et al.* (1990) found no significant difference between them. This is compatible with the results of the field trials made in Britain, discussed above, and with the results shown in Fig. 28. Accordingly, the reported higher incidences of *A. woodi* in the USA than in Britain are probably for reasons other than a difference between the genetic susceptibilities to infestation of individual American and British bees. Factors that control contact between migrating mites and young bees must determine the issue.

(b) Colony densities Some effects of colony densities on the levels of infestation in N. America may be discerned. The number of colonies in Mexico have doubled between 1974 and 1985 (Anon, 1982) and have probably increased considerably since 1955. This may be at least partially responsible

for the high levels of infestation there in comparatively recent times. In north-east Mexico, where infestation is particularly severe, with more than 30% of bees infested, Eischen *et al.* (1988) describe a scene where the land adjoining rivers and associated irrigation channels, in otherwise dry scrub, has become the focus of intensive agricultural activity, including beekeeping. Citrus is the most important crop in spring, and Eischen *et al.* say that "colony density then becomes high ... unfortunately this is when *A. woodi* populations are highest". In the USA, the mite was first detected in states where the average colony densities were about 0.95 colonies/km^2 compared with 0.49/km^2 in the rest (Bailey, 1985). Some of the highest infestations are in Florida, where the average density is about 2.4 colonies/km^2, which is the highest density of all and about double the world average. Even where regional supplies of nectar are good, localities can be overstocked, and infestations thereby aggravated, when many colonies are congregated into few sites (Table IX).

(c) Climate When brood-rearing is fairly continuous, or is only briefly interrupted, as in the warmer parts of Mexico and southern USA, young susceptible bees will nearly always be present. Accordingly, infestation can be expected to persist through the winters there, all other factors being equal, more readily than in colder climates.

II. OTHER *ACARAPIS* SPECIES

There are mites that spend their lives entirely on the outer surface of the bodies of bees but are morphologically almost identical with *A. woodi*. These mites were first found in Switzerland and the first species named was *Acarapis externus*. This species is localized in the area behind the head capsule of adult bees on the ventral side of the neck (Homann, 1933). External mites were then found on bees in Britain (Morison, 1931), but these were localized to the V-shaped groove between the mesoscutum and mesoscutellum (Fig. 31). Eggs, eggshells, larvae and larval skins usually lie in a contiguous row, with their long axes parallel to that of the groove and generally in its posterior region. These are of *Acarapis dorsalis*.

The evidence of several workers suggests constant biometrical differences between the three species of *Acarapis* (Brügger, 1936). The length of the terminal segments of the fourth leg of female mites of *A. woodi* and of *A. dorsalis* is between 7.3 and 7.8 μm but that of *A. externus* is about 11.8 μm; so *A. externus* seems well differentiated on this basis. The distance across the body between the two spiracles of the mite is about 13.7 μm in *A. woodi* and about 15–17 μm in *A. dorsalis* and *A. externus*. Although it may be justifiable

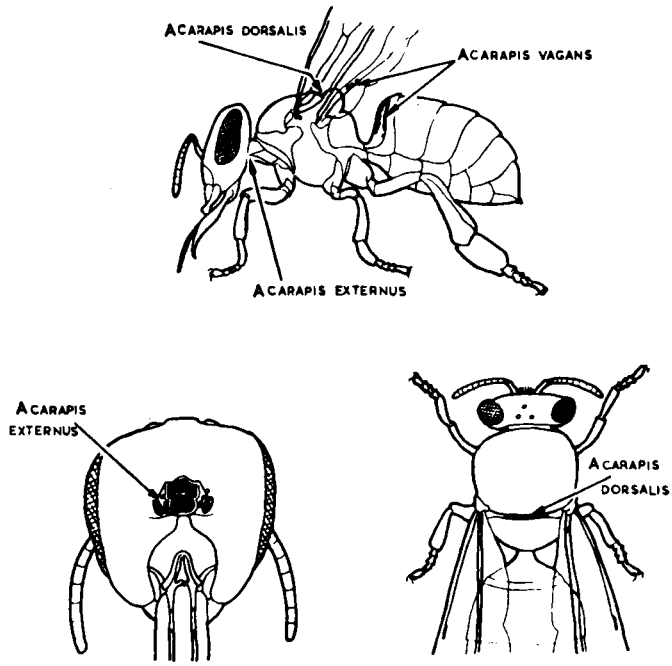

Figure 31 Location of species of *Acarapis* that live on external surface of adult bees. Lower left-hand diagram shows a rear view of the head. (After Morgenthaler.)

to distinguish between the mites by these measurements, some taxonomists have considered them insufficient for classifying the mites as separate species. Nevertheless, from the ecological aspect the mites are separate even if they cannot be separated satisfactorily on biometrical grounds. For example, in colonies infested with *A. externus* and observed for 5 years, mites were never found in the tracheae (Morison, 1931); and in more than 46 000 bees from colonies "heavily infested" with external mites, none were found in the tracheae (Borchert, 1929). No correlation has been found between the proportions of bees infested with different types of mite in colonies infested with all three varieties; and colonies have been found infested with two or only one of the three varieties (Brügger, 1936). All these observations strongly suggest that the mites are specifically associated with the restricted localities on the body of the bee where they are found, and that they are distinct species.

A third species of external mite, *Acarapis vagans*, has been proposed. It lives primarily near the roots of the hind wings of bees (Fig. 31); but in severe infestations the forewing, propodeum and first abdominal segment are also occupied, and they spread all over the body of drones. *A. vagans* seems well

differentiated from *A. woodi* on biometrical grounds, but it is not clearly separated from *A. externus* or *A. dorsalis* (Schneider, 1941), and has been relegated to *nomen dubium* by Delfinado-Baker and Baker (1982).

There is little information about the life cycle of the external mites. Eggs of *A. externus* are laid on bees about the second day after they emerge in severely infested colonies. The eggs hatch after 4 days and male larvae become adult after 2 or 3 days and female larvae after 4 days. The whole life cycle, therefore, takes about 10 days for a female, which is about 4 days less than that of *A. woodi* (Brügger, 1936). The life cycle of *A. dorsalis* takes about 11 days (Royce *et al.*, 1988). All stages of *A. dorsalis* die on dead bees after about 3 days (Morison, 1931). External mites apparently feed on the haemolymph of adult bees, like *A. woodi*, since they take up congo red that has been injected into the bodies of infested bees (Örösi-Pal, 1934). Their pathogenicity for bees has never been investigated.

Examination of dead bees in winter and flying bees in summer, both from the same colonies, has shown that the percentage of bees infested with *A. dorsalis* and *A. externus* is highest in winter and lowest about midsummer (Brügger, 1936; Homann, 1933; Clinch, 1976). The youngest bees are the most susceptible. Of 40 newly emerged bees introduced to a colony, 31 were found infested with *A. externus* 2 weeks later, but only 6 out of 20 bees introduced when 4 days old and 1 out of 17 bees introduced when 6 days old became infested (Brügger, 1936). Colonies severely infested with *A. vagans* were found to be mostly queenless (Schneider, 1941); and few external mites occur in normal colonies. In all these respects external mites resemble *A. woodi*.

Morgenthaler (1930) found *A. externus* throughout Switzerland, whereas *A. woodi* was more localized. *A. dorsalis* was commoner than *A. externus* although colonies with *A. externus* usually had a higher percentage of infested bees than colonies with *A. dorsalis*. External mites have been found in Scandinavia, Australia and New Zealand (countries where *A. woodi* has not been found) as well as in Europe, USSR, North and South America and Africa. There is no obvious explanation for the wider geographical distribution of *A. dorsalis* and, to a lesser extent, of *A. externus*, than that of *A. woodi*. There is some evidence that *A. dorsalis* is less restricted to young bees when it migrates, and it may breed more profusely in winter than *A. woodi*, which might account for its wide occurrence (Brügger, 1936). *A. externus* and *A. dorsalis* have shorter life cycles than *A. woodi*, which may help them to survive better than the latter by enabling them to migrate sooner to young bees. However, neither *A. dorsalis* nor *A. externus* have been reported from India even though mites resembling *A. woodi* occur there in *Apis cerana*.

III. *VARROA JACOBSONI*

This mite (Fig. 42f) was described by Oudemans (1904) who first recognized it in the brood cells of drone larvae of *Apis cerana* in Java. It was found on *A. cerana* in Japan in 1909 (Crane, 1984) and was identified for the first time on *Apis mellifera*, also in Japan, in 1958 (Mikawa, 1986). However, it has almost certainly parasitized the European bee there since 1915 (Takeuchi and Sakai, 1986), and perhaps even earlier because the European honey bee was taken to Japan in 1876 (Tanabe and Tamaki, 1986).

A. *Signs and Diagnosis*

The female mite is a dark red-brown and is almost 1.5 mm in diameter. It is the only common parasite of honey bees that can be seen with the naked eye and identified with a hand lens. This has helped to earn it unusual attention and notoriety, especially after it was found in colonies of *A. mellifera* in Europe about 1970.

The mite reproduces mainly on drone pupae of *A. cerana* (De Jong, 1988), and on worker pupae of *A. mellifera*, although it prefers the drones. Live mites usually occur inside sealed brood cells or are partly hidden between the abdominal segments of adult bees so, in spite of their size, they are not easily noticed by beekeepers. Dead mites, which fall from adult bees, can be seen fairly readily in debris from the hive floor. This debris may be collected conveniently on sheets of white paper, which are best left for some weeks, or even throughout the winter, before examination. Mites can be separated from wax particles and other debris by extraction in alcohol, on which they float (Ruttner and Ritter, 1980). Collection can be accelerated by blowing the smoke from 2 or 3 g of smouldering pipe tobacco from a hive-smoker into the hive entrance during the evening in late autumn or early spring, preferably when there are few unsealed bee larvae and no freshly collected nectar. The hive entrance is then closed and the floor debris removed early the next morning. Many of the mites on adult bees are killed by the smoke and fall to the floor (Ruijter and Eijnde, 1984).

The nymphal stages of the mite pierce the cuticle of pupae and feed on the haemolymph. They diminish the weight of the emergent adult bee in proportion to their number (De Jong *et al.*, 1982b). Although most bees from parasitized pupae appear normal, they start to fly a few days sooner than bees from uninfested pupae, some failing to return after their first flights (Schneider and Drescher, 1987). Perhaps more importantly, their lives are shortened especially in periods of dearth and to the greatest degree in autumn and winter (Kovac and Crailsheim, 1988).

Much damage, at least in Europe, is due to acute paralysis virus, which commonly infects bees without causing apparent harm. However, mites activate the virus when they attack these bees (Chapter 3, III) and then transmit it to kill further individuals. These include larvae and pupae which show symptoms, especially in severely infested colonies, that have been confused with those of European foulbrood or American foulbrood. Some parasitized pupae produce adults with deformed wings (Fig. 42i). This damage is invariably caused by deformed wing virus which is also transmitted by the mite (Chapter 3, IV.). The bees with deformed wings soon die. *Varroa jacobsoni* also acts as a vector for sacbrood and black queen cell viruses (Ball, 1989). It may do so less frequently than for acute and deformed wing viruses, but it clearly has a formidable capacity to transmit a range of unrelated viruses.

Mites attached to apparently normal adult bees pierce the body wall between the abdominal segments and feed on the haemolymph. They prefer the youngest adult bees (Kraus *et al.*, 1986), and they cannot survive for more than a day or so when separated from their hosts (De Jong *et al.*, 1982a).

B. *Multiplication*

The female lays between 1 and 12 eggs in a brood cell of *A. mellifera*, preferring that of a drone just before it is capped. The first egg is male (Rehm and Ritter, 1989) and is laid about 60 h after the cell is capped; the next eggs, usually female, are laid at 30 h intervals. Some mites eventually reinvade brood cells and reproduce for a second and, rarely, for a third time. Male mites, which are smaller and paler than females, die soon after mating within the sealed brood cells.

As many as 21 mature females have been found invading one drone cell. However, multiplication is restricted, particularly on worker pupae, because only the first few offspring of one female mite have time to mature and mate before the adult bee emerges about 12 days after the cell is capped. Furthermore, although most eggs laid on drone pupae are viable, up to half the female mites on worker pupae lay no viable eggs or no eggs at all (Camazine, 1988). On bees in Uruguay, 60 to 90% of females were infertile (Ruttner *et al.*, 1984). The reasons for such infertility are unknown but are obviously of great importance. They may well be related to the fact that female mites each produce fewer female offspring when more than one invade one worker or drone cell, and do so in proportion to their number (Fuchs and Langenbach, 1989). In some races of honey bee, e.g. *A. mellifera capensis* from South Africa, the post-capping stage of worker pupae is so short that fewer than half of those that become infested produce adult female mites (Ramirez and Otis, 1986).

"Africanized" bees in S. America (bees believed to be descended from

A. mellifera adansoni taken to S. America in 1956 (Crane, 1988) and feared because of their aggressiveness by many beekeepers) have an 11 day mean post-capping period for worker pupae. This allows only one or two female offspring of *V. jacobsoni* per female mite (Camazine, 1988), whereas mites on European honey bees, with a mean post-capping period of 12 days, can each produce up to three (Rehm and Ritter, 1989; Schulz, 1984). Mites on drone pupae, whether European or African, can each produce an average of about three female offspring. Small differences between these rates can lead to enormous differences between the numbers of mites produced in a few generations. Assuming uninterrupted multiplication for 4 months, which is well within the bounds of possibility, a rate of multiplication of 1.2 would produce only about six female mites from one female, whereas rates of 1.7 and 2.7 would produce about 200 and 20 000 mites respectively.

Camazine (1988) suggests that these reproductive rates can explain the difference between the multiplication of *V. jacobsoni* in S. America, where the mite has usually been reported to have slight or even negligible effects, and that in Europe, where it is usually considered to be very destructive (De Jong *et al.*, 1984). Camazine estimates that when there is no drone brood the rate of replacement of female mites on European bees remains above 1.0, i.e. the mites multiply, whereas on African bees it falls below 1.0, i.e. the mite population declines.

By contrast with *A. mellifera*, colonies of *A. cerana* seem under no threat from *V. jacobsoni*. Apart from the fact that the mite attacks few worker larvae of *A. cerana*, the adult workers of this species remove and kill almost all the mites from their bodies and from the brood whereas workers of *A. mellifera* remove very few (Peng *et al.*, 1987). According to Sasaki (1989), reproduction of mites on *A. cerana* in Japan is limited mainly by the usually very small number of drone cells: very few adult female mites develop on worker larvae, and they do so only at the periphery of the brood-nest where larvae are coolest and so have a period of development long enough for some mites on them to reach maturity.

C. Spread within Colonies

1. Rate of Spread

The spread of *V. jacobsoni* is widely believed to be relentless within colonies of European honey bees once they become infested. Untreated colonies are said to die about 3 or 4 years after the mites have been detected, usually during the winter (De Jong *et al.*, 1982a; Koeniger and Fuchs, 1989). However, few data are available. Most evidence seems to be circumstantial. Many colonies can be expected to fail within 3 or 4 years for a great variety

of reasons, so *V. jacobsoni* may often be a secondary and sometimes relatively unimportant factor.

Romaniuk and Duk (1983) observed that infestation in 16 untreated colonies of an apiary of 60 colonies in Poland increased from about 5% of the bees in spring to about 16% in August and 20% in September. Assuming only ten cycles of reproduction of mites in one season—and there is usually enough brood for about 15 (Ribbands, 1953)—the net reproduction rate of the mite was only about 1.15, which is much less than the rates that seem possible (Section B.).

2. Factors Influencing Spread

Apart from the circumstances that restrict multiplication of mites, already discussed in Section B, there are other factors that probably inhibit the spread of infestation. Many mites on adult bees will become increasingly separated from the brood and lost in the field on the bees that eventually die there when foraging increases. Perhaps equally important, the post-capping stage of worker larvae is shortened by up to 24 h during nectar flows (Ribbands, 1953), which will prevent many mites from completing their development. Also, many mites are lost on adult bees that die in winter. Reported losses of between 3 and 38 mites per day throughout the European winter (Müller, 1987) amount to some 5000 mites per colony, which would be a substantial proportion in a colony with 20% of its bees infested; and whereas infestation remains high in the south of Russia, most mites die during the winter in colonies kept in northern regions (Poltev, 1978).

Factors contrary to these can of course be expected to favour the spread of infestation. De Jong *et al.* (1984) observed that colonies suffering restrictions to their normal activities were most susceptible to severe infestations, and the same applied to *Euvarroa sinhai* (Table VIII) infesting *Apis florea*. This is characteristic of certain other enzootic parasites of honey bees, notably *Acarapis woodi* (Section I.C.) and chronic paralysis virus (Chapter 3, I.E.), which spread quickest when the restriction of normal activities of colonies, particularly foraging, brings infested individuals into closest contact with susceptible young bees. Warmer or shorter winters, as in southern compared with northern Russia mentioned above, will maintain infestations by extending periods of brood-rearing, often in relatively poor foraging conditions, so facilitating access for mites to young bees and shortening the interval when mites have only ageing adult bees on which to survive. In another likely example of this, the incidence of *V. jacobsoni* becomes especially high in southern France where many beekeepers, who provide bees primarily for the pollination of orchards and melons, winter their colonies on the mild Mediterranean coast in very high concentrations (Robaux, 1988).

3. Colony Densities

Ritter and Leclercq (1987) and Ritter (1988a) describe how infestation increased only a little in slightly infested colonies when these were taken to infested areas where there were few colonies per unit area (low colony densities) but increased greatly when they were taken to areas of high colony densities. They considered that the difference was due to different rates at which lost infested bees drifted, according to the colony density, into the introduced colonies. Drifting bees can of course take mites into uninfested colonies and so establish a new infestation, or tend to equalize infestations between already infested colonies. However, drifting will not control the average level to which infestation rises or falls generally within a region. As already discussed, this level probably depends largely on environmental factors that affect the performance of colonies. The foraging potential of the colonies in the studies under discussion would have been greater, due to less competition, in the low density areas than in the high density areas.

In Japan, the mite was believed not to have spread much among and within colonies of *A. mellifera* until about 1967 (Mikawa, 1986). Indeed, although *V. jacobsoni* has almost certainly infested European honey bees there since the early part of the century, it was not until 1970 to 1975 that its incidence seemed to rise markedly (Takeuchi and Sakai, 1986), and this was at a time when colony densities were rapidly approaching their highest known value, with the honey yield per colony—i.e. the foraging activity of individual colonies—much diminished, even though the total yield of honey was more than that of previous decades (Fig. 32).

In many parts of central Europe, where most accounts of severe damage by *V. jacobsoni* have arisen, average colony densities approach ten colonies per km^2 (Crane, 1975; Anon, 1982), which is about ten times the world average. This and cold winters, and perhaps comparatively high incidences of the viruses that the mite transmits (Section A.), may account for the severe effects of infestation in Europe compared with their seeming absence in more productive areas such as Uruguay (Ruttner *et al.*, 1984) and Brazil (De Jong *et al.*, 1984). In Brazil, there were lower levels of infestation in feral colonies than in beekeepers' colonies which were probably at higher average densities, and clustered feral colonies were significantly more infested than isolated ones (Goncalves *et al.*, 1982).

When European bees were taken from the Ukraine to Primorsk (the far eastern province of the USSR) early this century, *Apis cerana* and probably *V. jacobsoni* were native there (Crane, 1988). The European bees became so renowned for their productivity that they were re-imported to European Russia about half a century later with the reputation of being superior strains, even though they were by then infested with *V. jacobsoni* (Crane, 1978).

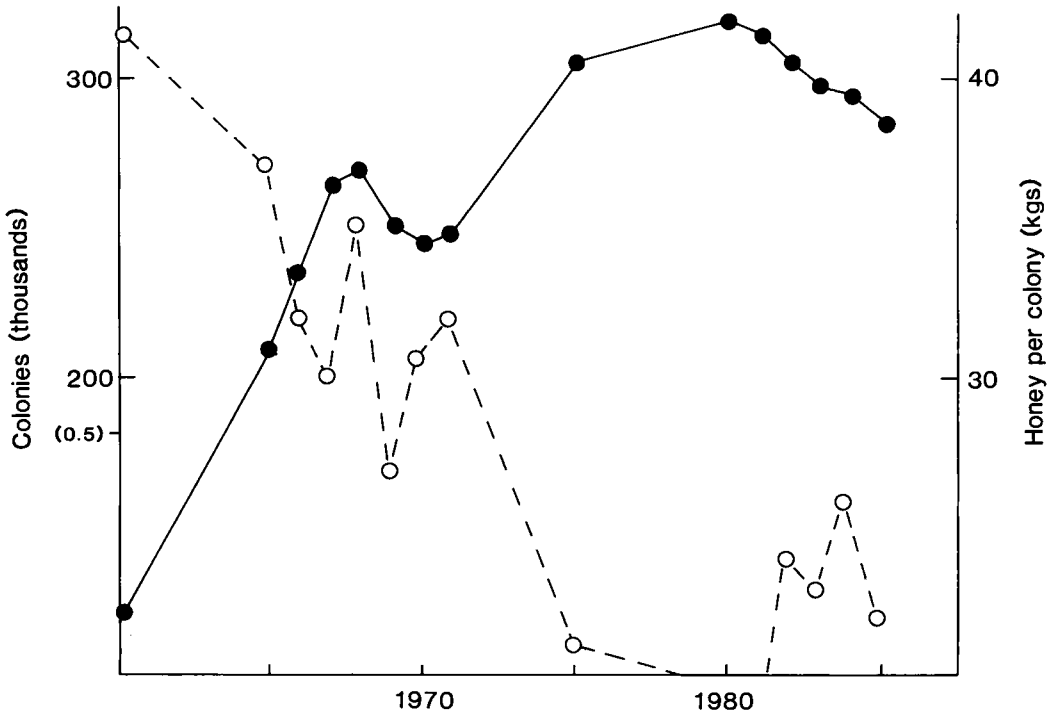

Figure 32 The numbers of colonies (●) and the honey yield per colony (○) in Japan. (0.5); 0.5 colonies/km^2. (From Anon, 1986b.)

Primorsk may simply be a region much more favourable for bees than central Europe because the colony density is low relative to the excellent sources of nectar. These are mainly lime forests, which have given an average annual yield of more than 100 kg of honey per colony over a 10 year period (Alpatov, 1976). There is no evidence that the bees from Primorsk are intrinsically more productive or resistant to *V. jacobsoni* than those of central Europe, but they are little affected by the mite when they are in optimal environmental conditions.

To sum up, the most favourable circumstances for the survival of enzootically infested colonies of European bees in temperate climates seem to be good summer nectar-flows followed by winters hard enough to kill off most of the few remaining infested individuals, and the worst seem to be poor nectar-flows, or too many competing colonies, followed by winters hard enough to kill severely infested colonies.

Environmental factors would seem to affect the outcome of infestation more than is popularly supposed. Kovac and Crailsheim (1988), who observed considerable seasonal differences between the effects of infestations of pupae on the life-spans of the resulting adult bees, concluded that "... climate, population and food conditions ... influence the damage".

D. Occurrence

Varroa jacobsoni is distributed widely on *A. mellifera* in the Old and New Worlds, and on both *A. mellifera* and *A. cerana* in Asia and the Far East. It has not been reported from Africa south of the Sahara or from Australia, Ceylon, New Zealand, the British Isles, Norway or Sweden (Bradbear, 1988). Its reported absence from some mainland areas of North and South America cannot be regarded with confidence in view of its presence in the USA, Brazil, Argentina and other S. American areas.

The general opinion is that the mite was taken to European Russia (De Jong *et al.*, 1982a) sometime before 1965 and from there to central Europe about 1970. Only from that time did it excite great attention, first in western USSR about 1965, then Bulgaria about 1967, Rumania in 1975, West Germany in 1977, and in other European, Middle Eastern and North African countries about the same time as in W.Germany, or very shortly afterwards. The impression is of an inexorable and very rapid spread westwards, although it is impossible to be sure that the mite had not been present some time before the dates given. The notoriety of the mite as a serious pathogen of *A. mellifera* only shortly preceded its discovery in Europe. This was when it was found in colonies of *A. mellifera* that had been taken to the Philippines and other tropical regions of Asia (Delfinado, 1963), where the European honey bee is not native and has often failed. Its failure has been attributed

largely to infestation by *V. jacobsoni* and *Tropilaelaps clareae* (Morse, 1969).

V. jacobsoni is believed to have arrived in S. America on queens of *A. mellifera* that were taken to Paraguay by Japanese beekeepers in 1971 (De Jong *et al.*, 1984). The origin of the infestation in the USA, first found there in September 1987 (Needham, 1988), is unknown. It had been detected in 18 US states by 1988.

Delfinado-Baker and Houck (1989) found significant differences between the body sizes and the numbers and positions of various setae of *V. jacobsoni*, sufficient to identify three biotypes:

A, from *A. mellifera* of European origin,
B, from *A. cerana*, and
C, from Africanized (Brazilian) strains of *A. mellifera*.

They concluded that the biotypes differed in virulence, corresponding with the different degrees of damage with which they are associated. There could be other interpretations of these associations (Sections B., C.3.), but the biometrical observations may well give clues about the origins and interrelationships of infestations. In particular, the North American types probably came from those in S. America, which in turn seem closer to mites from Asian strains of *A. mellifera* than to those from European strains. Mites from European bees most closely resemble those from *A. cerana*.

IV. *TROPILAELAPS CLAREAE*

Several other mite species that live externally on the brood or adults of honey bees have been reported from the Far East and South East Asia (Table VIII). The commonest is *Tropilaelaps clareae* (Delfinado and Baker, 1961) (Fig. 42e). This mite feeds on the haemolymph of bee larvae, preferring those of drones but invading cells of worker larvae much more readily than *Varroa jacobsoni*. It cannot pierce the body wall of adult bees, but it may steal food from their mouths, rather like *Braula coeca* (Chapter 8, I.B.). However, it cannot survive in bee colonies outside their brood cells for more than one or two days. It moves with great agility on the combs, probably evading the attention of the adult bees of *Apis mellifera* even better than *V. jacobsoni*; but the worker bees often detect infested cells, break them open and remove or partly remove the pupae. Adults emerging from infested cells sometimes have malformed wings (Ritter and Schneider-Ritter, 1988).

According to Ritter and Schneider-Ritter (1988), about a third of female mites fail to reproduce: of the rest, most lay only one female and one male egg, about a third lay two female and two male eggs, and about 3% lay three

eggs of each sex. They estimate a net reproductive rate of only about 1.02, so other factors must lead the mite to spread eventually to exclude *V. jacobsoni* in mixed infestations and then overwhelm colonies of *A. mellifera* in S.E. Asia. An important reason could be the shorter time of development of *T. clareae*, which would give it the advantage over *V. jacobsoni* when the post-capping stage of bee larvae is shortened during nectar-flows (Section C.2.). Other factors may be the greater preference shown by *T. clareae* for worker larvae than by *V. jacobsoni* and the need by *T. clareae* perpetually to infest brood, accordingly losing few or no individuals on adult bees that die in the field.

According to Griffiths (1988), the non-specialized features of the mite and the morphology of its mouth-parts characterize the species more as a predator than a parasite. Its wide range of bee hosts and the possibility that its adults may be able to feed on other hosts, even the young of small mammals, seems to justify this view. The Chinese are alleged to have seen it on rats, and the type species (Delfinado and Baker, 1961) apparently was taken from a rat nesting near bee colonies in the Philippines. All of this implies a formidable capacity for survival and spread.

De Jong *et al.* (1982a) observed that queenless colonies of *A. dorsata* with laying workers (workers in queenless colonies that lay unfertilized and, therefore, drone eggs) became most severely infested by *T. clareae*. Such colonies are, of course, dwindling and doomed anyway. The small and diminishing amount of brood probably becomes noticeably more damaged than in flourishing colonies.

Originally reported not to infest colonies of *A. cerana* (De Jong *et al.*, 1982a), it has since been reported as doing so (Table VIII). Presumably the Asian bees are much more able to suppress the spread of *T. clareae* than European bees, but the reasons for this are not known.

The inability of *T. clareae* to survive in broodless colonies precludes its survival over the winter in temperate zones (Woyke, 1985b), but it seems to be a potential threat to beekeeping with *A. mellifera* in tropical and sub-tropical regions. It has, for instance, been found infesting 25 to 50% of over 5000 colonies of *A. mellifera* which were apparently free of *V. jacobsoni*, *A. woodi*, American and European foulbrood, and sacbrood in the Punjab (Chahal *et al.*, 1986). So far it has been reported only from the Indian sub-continent, China and S.E. Asia (Bradbear, 1988).

8
INSECT AND NEMATODE PARASITES

I. DIPTERA

A. *Larvae*

The larvae of some species of fly parasitize adult honey bees, which are then said to be suffering from "apimyiasis". The adult female fly of *Senotainia tricuspis*, a common European species, waits near entrances of beehives and swoops down on flying bees to deposit a tiny, newly-hatched larva on their backs, usually on the joint between head and thorax. Flies may contain 700–800 larvae and can deposit 1 per bee every 6–10 seconds (Boiko, 1958). The flies are not specifically associated with honey bees: two species of *Bombus* were found parasitized in an area where 40% of honeybees were infected (Boiko, 1948).

The larva is armed with piercing mandibles which enable it to penetrate the intersegmental membrane where it is deposited, and to enter the haemolymph of its host. It grows in the abdomen or, more usually, the thorax of the bee, but does not feed on the solid tissue while its host is alive (Simmintzis and Fiasson, 1949).

When the bee dies, the fly larva eats the thoracic muscles, moves to the abdomen and eats the soft tissue there, and finally leaves the shell of its host to pupate. If it encounters another dead bee, however, it will eat the bee's contents first and then pupate. Larvae do not enter living bees after leaving their dead host (Giordani, 1955).

Larvae pupate in the soil and emerge as adults after 7–16 days; or in the following year, about July in the northern hemisphere, after a winter period of diapause.

Lesions to thoracic muscles of bees parasitized by *S. tricuspis* and *Melaloncha ronnai*, a Brazilian species, have been reported, but abdominal myiasis caused by either fly seemed to cause no damage (Guilhon, 1950). *M. ronnai* has been

said to paralyse its host in which it also pupates (Ronna, 1936); but it is a member of the Phoridae family and these "hump-backed flies" are generally scavengers on dead bees (Milne, 1951). They parasitize moribund bees, and Guilhon (1950) described "pseudo-myiasis" caused by post-mortem infestation by numerous species of Diptera. However, Örösi-Pal (1938a) cites examples of them being true parasites. *Borophaga incrassata*, another of the Phoridae, has been said to lay eggs in bee larvae that continued to develop and even became sealed over, often with their heads to the bases of their cells, and then died (Paillot *et al.*, 1944). Severely affected brood has been described as resembling foul-brood or chilled brood.

Drosophila busckii has been found in the thoraces of a few bees (Mages, 1956). The fly resembles *S. tricuspis* but apparently develops entirely within the bee, metamorphosing within the thorax or the abdomen.

Peak infection by *S. tricuspis* occurs in August in southern France, and only hives exposed to bright sunlight are affected (Simmintzis and Fiasson, 1951). But the proportion of parasitized bees usually seems low: out of 40 000 bees examined in July, August and September, only 69 were found parasitized (Rousseau, 1953).

There are very conflicting statements about damage to colonies by apimyiasis. *Sarcophaga surrubea* has been considered damaging to honey bees in North and South America (Braun, 1957). *S. tricuspis*, when first discovered in 1929 by Angelloz-Nicoud and for many years afterwards, was thought to cause devastating losses: inability to fly, paralysis and crawling in front of the hive, all being given as signs of infection (Simmintzis, 1958). Methods of control were advocated, legislation contemplated and even enforced in some European countries. Severe damage by *S. tricuspis* has been said to occur in the USSR, with 70 or 80% of bees lost from colonies in the Ukraine and "mass mortality" near the lower Dnieper (Sukhoruka, 1975). Yet others report no sign of damage, even in colonies with 80% of bees infected (Giordani, 1955).

Diseases caused by pathogens that are less readily detected than fly larvae are probably often mistaken for apimyiasis, especially when many bees are parasitized.

S. tricuspis has been reported mainly from France and Italy. It also occurs in North Africa (Mathis, 1957) and Australia (Roff, 1960). *Rondaniooestrus apivorus* causes apimyiasis in South Africa (Milne, 1951).

B. *Adults*

Braula coeca is associated specifically with honey bees. Eggs (Figs 33, 42h) are laid on the inner side of the cappings, and sometimes the walls, of cells full of honey. Subsequent development is entirely beneath the cappings of

honey cells and not among brood cells. The grub-like larva (Fig. 33) makes a tunnel of wax fragments which it gnaws from the cappings. This tunnel, traversing several cells, is very thin at first but expands as the larva grows. Larvae presumably obtain protein food from pollen which, together with wax, occurs in their intestines. Micro-organisms within the epithelial cells of the mid-intestine may help to digest wax. When fully grown, the larva pupates within its tunnel. The adult emerges and finds its way to the bodies of worker or queen bees and occasionally to drones. Often many collect on the queen at once, probably because she is the most permanent member of the colony rather than especially attractive. Daily collections from a single queen have totalled 371, about 30 being found at any one time. *B. coeca* feeds by moving from its usual place on the constriction between the thorax and abdomen of

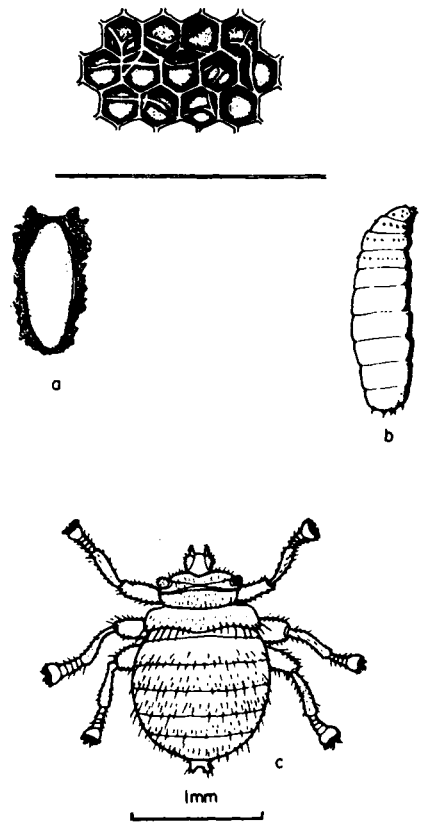

Figure 33 *Braula coeca*. Above: underside of honeycomb cappings showing wax tunnels of larvae of *Braula coeca*; below: (a) egg, (b) larva (c) adult. (Partly after Imms.)

the bee to the head when its host is about to feed, and takes up a position on the open mandibles and labium. It reaches into the cavity at the base of the extended glossa of the bee near to the opening of the duct of the salivary glands to obtain food, possibly salivary gland secretion (Imms, 1942).

B. coeca is usually described as an inquiline, and therefore not harmful, but it is possible that severe infestations decrease the efficiency of queens.

Örösi-Pal (1966) mentions several species and sub-species of *Braula* and describes three groups represented by (1) *B. coeca*, (2) *B. schmitzi* and (3) *B. pretoriensis*, the latter being the type in Africa.

II. COLEOPTERA

Larvae of beetles (*Meloë* spp.), first called *Pediculus apis* by Linnaeus and now known as triungulin larvae, hatch from their eggs on the soil, climb onto open flowers and attach themselves to the bodies of aculeate Hymenoptera. The active larva, about 1 mm long, drops on to the egg in the nest of solitary bees to be sealed up with the store of pollen and honey. It eats the egg, changes to the grub-like larvae and then, after eating the stored food, pupates and emerges as an adult oil beetle a month later or more usually after hibernating through the winter.

Larvae of *M. cicatricosus* have been said to find and eat the eggs of honey bees, to turn into grubs that look like bee larvae and, astonishingly, to be fed by the adult bees (Seltner, 1950).

Triungulin larvae have been reported to burrow in the joints of the abdomen and thoraces of adult bees, which then die with convulsive movements. Hundreds of such bees have been seen dying on the ground of Russian apiaries (Beljavsky, 1933).

Larvae of *M. variegatus* on bees injected with congo red took up the dye, which suggests that they pierce the body wall and suck the blood of the bee (Örösi-Pal, 1937a). *M. proscarabaeus*, when tested in the same way, did not take up the dye but were seen to consume honey from the comb.

M. variegatus occurs in North America, Russia, Europe and Britain, but *M. proscarabaeus* is the common species in Britain and does not seem to harm bees.

III. STREPSIPTERA ("STYLOPS")

Species of Strepsiptera have been found parasitizing honey bees (Johnsen, 1953). The insects have initial larval forms that look like triungulins of oil

beetles, but they parasitize larvae of various species of Hymenoptera, living in their body cavities in a grub-like form and feeding on the haemolymph which diffuses through their body walls. The larvae continue to develop and emerge as adults, although they may be deformed: the pollen-collecting apparatus may be absent or the sting may be diminished in size.

Female stylops live permanently within their host, their head and genital apparatus protruding through the bee's cuticle. Male stylops are free-flying and mate with the female after alighting on the host insect. Ulrich (1964) doubts that stylops can complete their life cycle in honey bees, although the larvae are probably carried to the colony by foragers. Certainly, honey bees are rarely parasitized by stylops and damage is probably slight.

IV. LEPIDOPTERA

Wax moths usually destroy combs unoccupied by bees, but in warm climates the greater wax moth, *Galleria mellonella* often overwhelms small colonies. Occasionally, the lesser wax moth, *Achroia grisella*, and, less often, *G. mellonella*, chew away some of the cappings of sealed brood and the adult bees remove the rest to cause a condition known as bald-brood. The absence of cappings over pupae is of little consequence, but some of the pupae emerge with deformed legs and wings (Milne, 1942).

V. NEMATODES

Mermithid nematodes have occasionally been found in honey bee workers, drones and queens. They are 10–20 mm in length, very thin whitish worms that parasitize many insect species. Mature adult worms dwell in the soil and mate there. The eggs are laid on wet grass by the active female, or else the young larvae make their way there. Insects eat the grass, or take dew from it, and the eggs are ingested or the young larval nematodes penetrate the insect cuticle.

Queen honey bees probably receive nematode eggs that are brought in by bees collecting water. One queen whose ovaries contained no eggs was found to have an encapsulated nematode in her body cavity near the hind-gut and ovary (Kramer, 1902).

Nematodes probably have to go through a soil phase, so they are not likely to multiply and spread within bee colonies. Their natural hosts are probably ground-dwelling insects, including solitary bees and bumble-bees. However,

Vasiliadi (1970) reported some 60% of many hundreds of honey bees he examined to be parasitized by mermithids in low-lying regions of the USSR.

Mermis nigriscens has been found in honey bees in Switzerland and an *Agamermes* spp. was found in honey bees in Brazil (Toumanoff, 1951). *Mermis albicans* has been found in worker, queen and drone honey bees in Europe (Fyg, 1939; Paillot *et al.*, 1944) and larval mermithids were found in worker bees in eastern USA by Morse (1955).

9
DISORDERS OF UNCERTAIN ORIGIN AND NON-INFECTIOUS DISEASES

Several non-infectious disorders are often confused with infectious diseases. Dysentery and various kinds of poisoning are the commonest.

I. DYSENTERY AND POISONOUS SUGARS

By common usage among beekeepers, "dysentery" means defaecation by adult bees within or near the colony so that the comb and the hive entrance are visibly soiled with excrement (Fig. 41f). Bees accumulate material in their rectums when they are prevented from flying, so defaecation within the hive occurs mainly in late winter. Usually it is slight and of little consequence, but sometimes it is severe and associated with the rapid death of affected colonies. The bees die from a combination of suffocation and turmoil in their efforts to clean themselves and their combs of the excrement, and of associated pathogens.

Langstroth (1866) noticed that bees confined to their hives in damp conditions were apt to become dysenteric; he also considered that feeding bees on liquid honey in autumn or disturbing bees in winter were the prime causes of dysentery. Dadant (1890) and Root (1901) thought that dysentery was caused by dilute honey, fruit juices that beekeepers sometimes fed to bees, or by honey-dew, which is the excrement of aphids often collected by bees; and Phillips (1945) considered dextrins, which occur in honey-dew, caused dysentery. However, Lotmar (1934) showed that bees can digest dextrins as well as starch partly decomposed by boiling, and Alfonsus (1935) demonstrated that dysentery was not caused during winter when colonies were fed during the preceding autumn either on 15% dextrin in sucrose, honey-dew, strong sucrose solutions, or large amounts of pollen in sugar candy. He showed that dysentery was caused by feeding liquid honey, or

dilute or crystallized sucrose solutions. Dysentery occurs when the rectal contents of bees become about 30–40% of their total body weight (Alfonsus, 1935; Lotmar, 1951) and this is due to the accumulation of water, usually because there is too much water in the food.

Dysentery often occurs when honey stores become coarsely granulated: the bees cannot ingest the large crystals and the liquid contains too much water. Sometimes this dilute honey ferments and, although the yeasts and alcohols produced by the fermentation are probably harmless, the process produces even more water.

However, there is an important kind of dysentery, which, although ultimately the result of water accumulating in the rectum, is not because the food contains too much water, but because it contains certain toxins. The toxins, as yet unidentified chemically, are produced by the action of organic or inorganic acids on glucose or fructose, and they appear to upset the normal metabolic water balance within the bees. They shorten the lives of bees by 50% or more when they are the only source of carbohydrate food. The most striking sources of these toxins are acid-inverted sucrose and acid-hydrolysed starch which have frequently been marketed as sugars suitable for feeding to bees (Bailey, 1966).

Hydrolysed sucrose is commonly, but mistakenly, believed to be nutritionally better for bees than sucrose because of its similarity to honey, and it is also favoured because it can be used to make a candy that stays soft and suitable for feeding to bees. However, unless hydrolysed with enzymes, it quickly causes dysentery and kills bees. Honey that has been heated is similarly toxic for bees, because of the action of its natural acids on the glucose and fructose. Even honey that has been stored at ambient temperatures for several years causes dysentery and shortens the lives of bees compared with those fed on sucrose. Sucrose partially hydrolysed by boiling with cream of tartar or vinegar, recommended by many beekeeping manuals as the basis of a solid candy for feeding the bees, is also toxic for bees when compared with plain sucrose.

In view of these facts and of the toxicity of unrefined sugars, including semi-refined beet sugar, the only form of sugar that can be recommended unreservedly for feeding to bees is refined sucrose. Moreover, concentrated sucrose syrup is far less likely to crystallize after it has been stored in the comb by bees than natural honey, because it contains a much lower proportion of glucose (Simpson, 1952).

An extreme example of crystallization of honey was given by Greenway *et al.* (1975) who observed that honey largely composed of ivy nectar contained 70% glucose, whereas the average concentration of glucose in honey is only 40–50%. Because of its high glucose content, the ivy honey granulated quickly and then lost water by evaporation, unlike ordinary honey, which is hygroscopic even when granulated, because of its high fructose content. Many colonies

in Eire that had stores largely composed of ivy honey, died during the winter, apparently because they could not obtain sufficient water from their food.

II. PLANT POISONS

There seem to be several sources of poisonous nectars and pollens (Burnside and Vansell, 1936; Maurizio, 1945), although the poisoning of bees by them is not easy to verify, especially as incidents are frequently local and transient.

In the USA trouble arises from California buckeye (*Aesculus californica*). It has been estimated that 15 million acres of foraging area becomes virtually unavailable for bees for 6 weeks in early summer when buckeye, a species of horse-chestnut, is in bloom. Both pollen and nectar are injurious; saponin is believed to be the toxin. Adult bees become trembly and lose their hair, partly or wholly, because unaffected bees pull at them as if trying to eject them from the hive (cf. Paralysis, Chapter 3, I.A.1.). Young brood are killed, deformed pupae arise from sealed brood and queens are said to become drone-layers. The nectar of horse-chestnut contains 50–60% sugar and is therefore very attractive to bees and, since the tree is resistant to drought, it may at times be virtually the only source of nectar.

Honey and pollen from lime (*Tilia* spp.) may be toxic. Many hairless, dark, trembly honey bees (cf. Paralysis, Chapter 3, I.A.1.) together with solitary and bumble-bees with the same appearance, have been seen under lime trees in midsummer, and the honey bees seemed to have no known infectious disease according to Maurizio (1943), although bee viruses were virtually unknown at the time.

Honey-dew from lime is sometimes toxic, and its high content of the trisaccharide melezitose has been blamed, although bees are known to be able to digest the sugar; and honey-dew from *Tilia platyphylla*, which killed bees within 8 h, contained a soluble, heat-stable toxin (Butler, 1943), unlikely to have been melezitose. Possibly it was galactose and/or mannose, both of which occur in the nectar and honey-dew of lime and are toxic for bees (Barker, 1978). Honey-dew from conifers is said to poison bees sometimes, particularly in Europe, where the disease is called *Waldtrachtkrankheit*. However, samples of bees suffering from this disease have been found to be severely affected with chronic paralysis virus (Bailey, 1976; Chapter 3, I.A.1.).

The nectars of *Rhododendrum ponticum* L. and other *Rhododendrum* species contain andromedotoxin, which is poisonous for bees. Some species of *Ranunculus* (buttercups) have anemonal in their pollen which is also poisonous to bees. Buttercup pollen may be collected, particularly when cold weather delays cherries and dandelions, and resultant damage to bees has been

reported from Switzerland where it is called "Bettlach May disease".

Astragalus lentiginosus (spotted loco weed) is troublesome in the USA at times: pupae die and mummify, adults turn black and hairless, and queens are said to die frequently from poisoning. *Astragalus* species can accumulate toxic levels of selenium, but toxicity to bees may not be related to this (Barker, 1978).

Cyrilla racemiflora (southern leatherwood) is said to cause "purple brood" in southern United States: unsealed larvae turn purple and die in early summer.

In experiments, nectar or pollen from death camas (*Zygadenus venenosus*) killed honey bees within 2 days, but strangely enough the solitary bee *Andrena zygadeni* appears to forage exclusively on death camas blossom (Hitchcock, 1959).

There seems little doubt that there are sources of natural poisons for honey bees, although many causes of natural poisoning are often difficult to establish. In view of more recent knowledge of hitherto unknown virus diseases of bees (see Chapter 3), reports of bee poisoning from plants should be treated with reserve.

The most common feature of natural poisoning, whatever the origin, is that more usual sources of nectar or pollen have failed, usually because of drought or cold.

III. INSECTICIDES

Poisoning of bees with insecticides has become an important hazard, sometimes causing severe losses which may be confused with the effects of infectious diseases of adult bees and larvae. Fortunately, most manufacturers and users of insecticides are aware of the danger to beneficial insects, and efforts are made increasingly to develop insecticides that are selective for pests, either by reason of their chemical nature or by their method of application (Wilson *et al.*, 1980). An assessment of the relative toxicities to honey bees of a wide range of insecticides has been given by Stevenson *et al.* (1978).

IV. HEREDITARY FAULTS

Eggs that fail to hatch, larvae that fail to pupate and pupae that die of no apparent infection are sometimes decribed as "addled" and are believed to be suffering from hereditary faults.

About half the number of eggs from greatly inbred queens do not produce larvae, but disappear to give areas of unevenly developing brood. Mackensen (1951) predicted and demonstrated this. He believed that the eggs that disappeared were of diploid drones, i.e. had arisen from fertilized eggs, instead of being the usual haploid drones characteristic of all Hymenoptera. All the fertilized eggs were laid in worker cells, but half of them were believed to be homozygous at a sex-determining locus of a chromosome and therefore male, and they were believed not to be viable. This mechanism of sex-determination and the non-viability of diploid drones had been found earlier in the wasp *Habrobracon juglandis*. The fact that diploid male tissue could exist in honey bees was later demonstrated by Rothenbuhler (1957) in gynandromorph or mosaic bees. Then, Woyke (1962, 1963, 1965) successfully hatched in an incubator all the eggs from inbred queens that had each been mated to one drone and that were laying eggs of which only 50% seemed to hatch in colonies; and he reared them to maturity in the laboratory. Half were drones and, as they reflected the genotype of their father, they were presumed to be from fertilized eggs. Later, Woyke and Knytel (1966) showed that such drones indeed had the diploid complement of 32 chromosomes. Therefore, the larvae of diploid drones are viable but, as Woyke (1963) showed, they are detected in bee colonies as soon as they hatch and are immediately eaten by nurse bees.

Instances of queens producing eggs which all fail to hatch (Hitchcock, 1956) are inexplicable on the genetic grounds given above and the fault may not be hereditary. Most cases of "addled brood" described by Tarr (1937c) in Britain were sacbrood (Chapter 3, II.), once believed not to exist in Britain but now known to be very common. "Addled brood" due to hereditary faults is probably less common than was once believed. However, the failure of prepupae to shed their last larval skin, for whatever reason, could cause the same signs as sacbrood, so the only certain diagnosis of this disease is the detection of the virus.

V. DISEASES OF UNKNOWN ORIGIN

A. *The Isle of Wight Disease*

The Isle of Wight disease was alleged to affect adult bees and was said to have reached epidemic proportions in the British Isles on at least three occasions between 1905 and 1919. The main symptom usually given was very many bees crawling and dying on the ground outside their hives during their active season, often leading to the rapid and spectacular death of whole colonies, especially in the height of summer (Fig. 41f). The first major

outbreak was said to have been on the Isle of Wight in 1906. The disease was then believed to have spread to the mainland in the south of England in 1909 and, according to Herrod-Hempsall (1937), by 1918 "not a beekeeping district in Great Britain was free from scourge [and] ... eventually the parasite [*Acarapis woodi*] invaded Ireland as well as European countries". This was the common belief, and it was typified by a statement about *Acarapis woodi* issued by the American Beekeeping Federation saying: "This Isle of Wight disease is considered by apiculturists in the countries where it does exist to be far more serious than American foulbrood".

There is no doubt that some beekeepers lost most of their bees in the Isle of Wight in 1906, which, apparently, was the worst of two or three consecutive bad years. It was then assumed, however, without any evidence, that the cause was a new and very infectious disease. This idea was then promulgated by sensational but uninformative articles, in *The Standard*, a now defunct London morning paper, and in several provincial newspapers. The publicity, as usual, helped to fix the belief firmly in the public mind.

The first professional investigation was made by Imms (1907). He examined bees on the Isle of Wight which were said to have the disease and found they had "enlargement of the hind intestine" which Imms, who at the time seemed unfamiliar with bees, thought abnormal. His diagram, however, represents very clearly the intestine of any normal bee that has been long confined to the hive. Malden (1909), the next professional investigator to visit the Isle of Wight, pointed out that the intestines of healthy bees confined to hives for a few days very closely resembled those of diseased bees. He had accepted the idea that there was an infectious disease, however, and he obtained a colony, said to have Isle of Wight disease, and confined them in a "warm room" in a muslin cage on 17 June 1908. By 10 August, he said, they had ceased to fly; and the colony was dead by 26 October. To keep them for so long under such conditions, however, would have been difficult had the colony started in the best of health.

Malden examined minutely the anatomy of bees said to have the Isle of Wight disease, including their tracheae and air sacs, but all he found were more bacteria in the intestines of diseased bees than in those of healthy ones; he failed to show that these micro-organisms were pathogenic. Bullamore (1922) also pointed out that bees prevented from flying sometimes develop symptoms, described as crawling with bowel distension, which were indistinguishable from those alleged to be of the Isle of Wight disease. In 1906, according to newspaper accounts, there was a disastrous April for agriculture, with frost (−5°C in London on 2 May) and snow after a very early spring, which had been hot enough to draw crowds to the seaside resorts. This very unusual weather might have accounted for trouble with bees, which, being suddenly confined to their hives, possibly with freshly gathered nectar, may

well have become dysenteric (Section I.) and paralytic (Chapter 3, I.E).

There is no more recorded evidence about the disease in those early days in Britain. There are, however, descriptions of the death of numerous colonies between 1901 and 1905 in several other countries, including Italy, Brazil, Canada and the United States, and all the bees had symptoms exactly like those described for the Isle of Wight disease in Britain (Bullamore, 1922). One incident was the loss of 20 000 colonies in Utah, with the bees dropping to the ground, mounting blades of grass and twigs with great difficulty and then dying. Had this disaster occurred in Britain at the same time it would have been attributed unreservedly to the Isle of Wight disease. Subsequently, there have been further reports of large scale losses of bees in many parts of the world, particularly in Australia and South America, with bees crawling and dying in front of their hives and with no known parasite present in sufficient numbers to be the cause. Poisonous nectar or pollen was suspected, but the causes may well have been viruses or spiroplasmas. Whatever their causes these losses would certainly have been classified as the Isle of Wight disease by beekeepers in Britain during the early 1900s.

There are all kinds of possible reasons for the death of bees, apart from infections, and there is little doubt that bees dying of non-infectious diseases were often included in casualties attributed to the Isle of Wight disease. Imms (1907) found the most successful remedy was "feeding cane sugar" and in Cumberland, where the disease was said to be serious in 1915 and 1916, with between 5 and 20% of colonies "affected" according to a report of their beekeepers' association at the time, it was said that "1916 was a poor season, many colonies were insufficiently provided for winter, and sugar was practically unobtainable". It appears therefore, that starvation was often to blame for some losses included in casualties alleged to be due to the Isle of Wight disease.

So-called treatments for the disease must have killed numerous colonies. One official report said diseased bees were short of nitrogen, because their distended rectums contained much pollen. This followed the mistaken belief that adult bees usually did not need protein food and, when they did, that pollen was unsuitable; so it was recommended that all pollen combs should be removed in autumn and the colonies fed beef extract to make good their supposed nitrogen deficiency. This would certainly kill or seriously cripple any colony because pollen is essential for adult bees and beef extract is poisonous for them, mainly because of its salt content. The ruinous idea of removing so-called "pollen-clogged" combs persisted, however, and was widely practised for many years. Other remedies that were recommended were phenol, formalin, "Izal", sour milk, salt and other chemicals lethal to bees, all to be fed in syrup to ailing colonies and as preventives to healthy ones. Other reports describe colonies which clearly were crippled with

foulbrood; and poison sprays were certainly used, probably with less consideration than today for bees. Perusal of all the British bee journals from their beginnings until about the 1920s shows that many beekeepers eventually attributed all colony deaths that had no obvious cause to the Isle of Wight disease. Some beekeepers were sceptical; they pointed out that the symptoms were those of the fairly well-known disease called paralysis (Chapter 3, I.) for which there was no known cause, but which had been described from time to time at least half a century before the Isle of Wight disease. Very dark strains of bee had the reputation of being especially susceptible to the Isle of Wight disease and the type 2 syndrome of paralysis would not have weakened this belief. The final opinion of Rennie (1923), a co-discoverer of *Acarapis woodi*, who had much experience with bees said to have the Isle of Wight disease, was that "under the original and now quite properly discarded designation 'Isle of Wight disease' were included several maladies having analogous superficial symptoms".

The publicity had won long before, however: by about 1912 almost everyone had accepted the idea that the Isle of Wight disease was infectious and thought that only the identity of the one supposed infectious agent was needed. This encouraged a burst of activity which culminated in the discovery of *Acarapis woodi* in December 1919 by Rennie *et al.* (1921) who at first considered this mite to be the cause. Their own results did not support this, however; on the contrary they made it clear that *A. woodi* was widespread, occurring in many apparently normal colonies. Their report showed that many bees from both diseased and healthy stocks behaved and flew normally, even though they were infested with mites, some of them with pronounced blackening and hardening of their infested tracheae. Normal nectar- and pollen-gathering bees from stocks in which "crawling and other symptoms were well established" were found heavily infested "quite as badly as anything . . . observed in crawling bees". In fact "flying workers were frequently more heavily parasitized than were bees of the same stock which were unable to fly". This evidence shows that *A. woodi* was not obviously pathogenic and certainly could not have been causing the observed sickness which was considered to be the Isle of Wight disease. It seems the mite was then much as it is today: its only significant pathological effect being to shorten very slightly the life of bees, but usually causing no obvious sickness in spite of the abnormal appearance of infested tracheae (Chapter 7, I.A.). Why *A. woodi* became so firmly established as the cause of the Isle of Wight disease in the face of this evidence is hard to understand. It may have been partly because of the size of the parasite, its incidence, and the appearance of infested tracheae, which were startling; but its restricted habitat in the thoracic tracheae, which are neither easy to see without a special dissection technique, nor of obvious interest, make its late discovery understandable. Perhaps some

thought it was the last adult bee parasite that would be found and, as the other parasites known at the time, *Nosema apis* and *Malpighamoeba mellificae*, did not seem very dangerous, it was believed *A. woodi* must be the cause of Isle of Wight disease. This ignored the possibilities of other pathogens, especially of viruses, which we now know to cause diseases with symptoms resembling those reported to be of Isle of Wight disease.

The confusion of thought about *A. woodi* is illustrated by the account in the book by Herrod-Hempsall (1937), which perhaps best reflects the popular beliefs of those days. He stated that *A. woodi* spread from the Isle of Wight to European countries after 1918. Yet in the same account he wrote "there is little doubt that [*A. woodi*] has infested the honey bee in a number of countries for several centuries". His second statement is nearer the probable truth, which is that the mite has infested honey bees for several thousand millennia; it almost certainly has no other host and it is extremely closely adapted to lead a life intimately associated with honey bees. It is widespread and has been found in India and Africa as well as Europe, Russia and America. It was found in France and Switzerland in the winter of 1921–22 and even in Tula, south of Moscow in 1922, where, incidentally, it was reported not to be associated with any sign of disease (Perepelova, 1927). This was a remarkable rate of spread if the mite started from England, especially considering the difficulties there must have been in transporting bees during those times. *A. woodi* is unknown in New Zealand and Australia, but so it might be in Britain if we enjoyed their comparatively regular and abundant nectar-flows. For it is in these conditions that mites decrease in number, quite possibly to become extinct (Chapter 7, C.). And it is in the opposite circumstances, when colonies are having a lean time, that *A. woodi* multiplies and spreads. These were the seasonal conditions in which Rennie and his colleagues discovered the mite, and his colonies which had suffered the worst conditions developed the severest mite infestations. Mite infestations increase in colonies as a result of their poor circumstances: it then adds to these, occasionally being the last straw, but usually it dwindles dramatically when the environmental conditions for bees improve enough to make them forage actively (Chapter 7, I.C.).

A very important point, suggested by Fig. 29, is that there could have been up to ten times as many colonies in Britain during the early part of the 1900s as there were during the 1990s. This is not at all unlikely: such high colony densities continue today in some mainland European countries. Early authorities and popular writers on beekeeping in Britain were enthusiastic about its profitability—"... nothing will tend to greater profit than bees" (Cotton, 1842); and others, e.g. Cowan (1881), exhorted beekeepers to feed large amounts of sugar in order to maintain many large colonies. These writings, and the great change from skep-hives to modern equipment about

the turn of the century, may well have encouraged a surge of beekeeping that much over-populated the country with bees, so leading directly to high incidences of paralysis and of *A. woodi*, and probably other contagious pathogens (Chapters 3, I.E; 7, I.C. & E.).

There are no obvious reasons why the number of colonies has subsequently declined so much in Britain. Many beekeepers believe that sources of nectar have declined, but not to the extent that the number of colonies seems to have done. Beekeeping may have been discouraged by many factors: the changing economic scene of the twentieth century; the ending of special sugar rations for beekeepers after war periods; the growing imports of cheap honey; a declining agrarian population; and, not least, the fear induced by publicity about the Isle of Wight disease. Whatever the reasons were, colony numbers have decreased nearer to self-sufficient levels, and the incidences of their contagious parasites have correspondingly declined.

To sum up, modern evidence leads to the suspicion that the Isle of Wight disease was assumed to be the cause of all the losses for which there was no explanation at the time. A higher proportion of colonies were probably more infested with *A. woodi* than later, with proportionately more winter losses; but most of the crawling bees considered to be typical of the Isle of Wight disease were almost certainly suffering from paralysis, the cause of which remained unknown until some 50 years later. Whatever the details were, they were far removed from the popular concept of the Isle of Wight disease. This was epitomized in *The Times* (London, July 22 1970; page 3), which stated "... a bee-killing parasite, *Acarapis woodi*, ... broke out in the Isle of Wight and swept Britain in 1911. In 1912 the flowers blossomed to an almost bee-less spring". Apart from the unjustified assumption about *A. woodi*, such sensational accounts are manifestly untrue, as testified by beekeepers of the day who reported to the *British Bee Journal* (April and May 1912) as follows:

J.M.E., Scotland (p. 166) "Bees are in ideal condition this spring".
L.S.E., Northern England (p. 166) "... some fortunate individuals already report full supers".
F.S.E., Eastern England (p. 168) "Bees are going strong here".
W.W., Central England (p. 173) "Every tree is loaded with blossom and the bees are in full work ... I can never remember my bees in better condition in April".
W.S.W., Western England (p. 176) "Bees are very busy among the fruit blossom ... stocks have come through this winter better than I ever remember before".

Similar reports were made throughout the period (1905–1919) when the disease was alleged to be most prevalent.

B. *Other Undiagnosed Abnormalities*

1. Queen bees

Fyg (1964) made many meticulous studies of the abnormalities of adult queens in Switzerland. He observed that some queens lay a disproportionate number of drone (unfertilized) eggs for pathological reasons, and not because they are unmated or have exhausted their supply of spermatozoa. They have plenty of spermatozoa, although many of these are usually coiled singly in the spermatheca instead of lying normally in wavy bundles. This abnormal feature of the spermatozoa is not absolutely diagnostic: 17 out of 223 of these "drone-broody" queens had normal sperm. The epithelial cells of the spermatheca always seem to break down, however, and their nuclei contain round or oval inclusion bodies which are slightly refractile, take acid stains and are of varying size and number. Fyg suggested that the inclusion bodies are caused by a virus, but their great variability in size and their staining qualities seem to differentiate them from the inclusion bodies that have been recognized in known virus infections of other insects (Chapter 3). When drone-broody queens lay a great many drone eggs, many of the resulting larvae are neglected, because of the shortage of nurse bees, and they die.

Fyg also describes stones that consist mainly of concentric layers of uric acid, each attached with a stalk of tissue to the rectal epithelium, sometimes occurring in the rectums of queens. They vary in size, usually being about 1 mm in diameter, though some of over 2 mm in length have been found, and they may be yellow, reddish-brown, grey or brownish-black in colour. Bacteria almost always occur on the stalks of the epithelial tissue that lie within the stones. Only a small proportion of queens with stones have other recognized infections, so the cause of the stones remains unknown. In the worst cases the stones seem to exert pressure on the genital duct and hinder oviposition. Stones somewhat smaller than those in queens are also found occasionally in worker bees.

2. Worker bees

Various non-infectious abnormalities of worker bees have been recorded. Rare cases have been reported of pupae that die shortly before they are due to emerge with the cuticle of their heads and first pair of legs still unpigmented. Some propupae develop similarly when they are removed from their comb and incubated artificially. The cause is blockage of the first thoracic tracheae with the final moulted cuticle, which is not completely stripped off (Fyg, 1958). This presumably prevents enough oxygen reaching the tissues to allow the cuticle to darken normally, but the reason for the failure to moult properly is unknown. Fyg also found worker pupae with abdomens extraordinarily

retracted and sometimes with enormously distended heads. For unknown reasons their honey-stomachs and mid-guts have become enclosed in the thorax and head, and the abdomen is retracted by the hind-gut which is still attached to the last abdominal segment and the mid-gut.

A fibromatous-like tumour, thought to be of neural origin, has been observed in the anterior lower portion of the thorax of a worker bee (White, 1921); and giant vacuolated cells have been seen amongst the epithelial cells of the hind-gut of old overwintered bees (Örösi-Pal, 1937b). The giant cells were formed apparently by the fusion of several epithelial cells and averaged about 100 μm in diameter, but some were up to 0.5 mm long; 18% of bees in one colony were found to be affected.

Dark-brown crusts have been seen in place of contiguous epithelial cells of the mid-gut in adult bees (Giordani, 1956). The bees were partially paralysed but attempts to infect healthy bees with the diseased tissue failed (Amici and Vecchi, 1958).

Wille (1967) describes two degenerative changes, of unknown cause, of the muscle tissues of sick bees. One is associated with "sharp-edged crystalloids" within the degenerating muscle fibres. The changes all frequently occur with other well-known infections, but they are not specifically associated with any of these.

3. Larvae

Larvae of all ages sometimes die without any apparent infection. "Chilled brood" is then often diagnosed, the implication being that not enough adult bees were present to cover the brood and keep it warm when they clustered at night or in inclement weather. However, unsealed larvae can survive several days at room temperature (about 18°C) without food, so severe or prolonged chilling would be necessary to kill them.

PLATE SECTION:
Figures 34 to 42

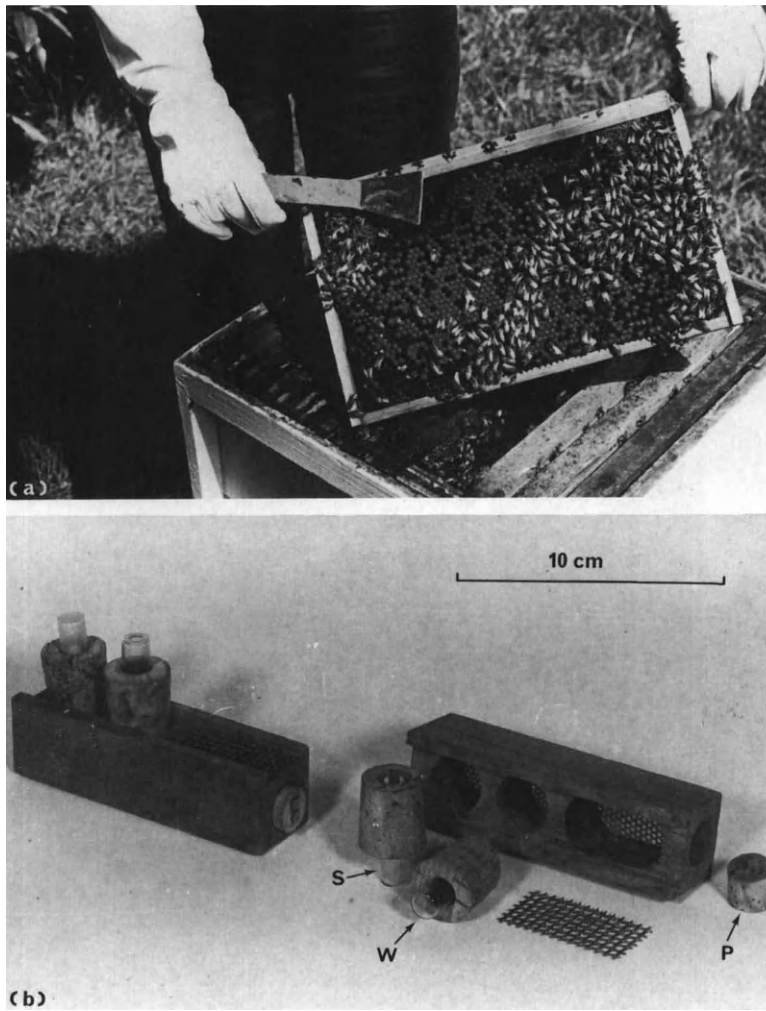

Figure 34 (a) Comb of bees and capped brood (Courtesy of Dr Ingrid H. Williams); (b) cages for laboratory tests, each suitable for up to 40 adult bees; S, W = vials containing strong sucrose solution and water respectively; P = cork with holes for pollen taken from bee combs.

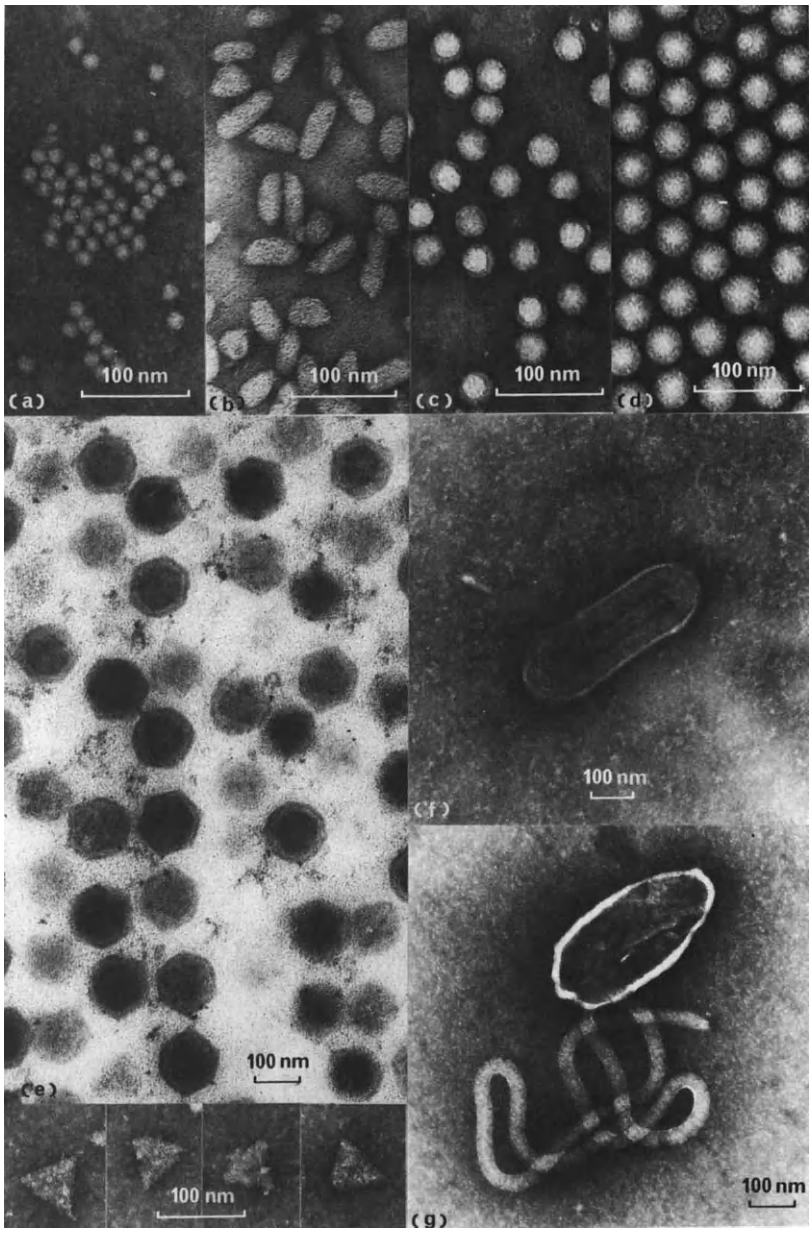

Figure 35 Electron micrographs of representative types of viruses from bees, (a) particles 17 nm in diameter (cloudy-wing particle virus, chronic bee-paralysis virus associate); (b) chronic bee-paralysis virus; (c) particles 30 nm in diameter (sacbrood, black queen-cell, acute bee-paralysis, Kashmir bee, deformed wing, Egypt bee, slow paralysis and Arkansas bee viruses); (d) particles 35 nm in diameter (Bee viruses X and Y); (e) *Apis* iridescent virus in ultrathin section of cytoplasm of adult fat-body cells; lower inset: trisymmetrons of sub-units that form the outer shell of the virus particles; (f) filamentous virus particle; (g) filamentous virus particle with ruptured envelope releasing the single flexuous rod, or nucleocapsid, which contains DNA and measures 3000 nm × 40 nm. All stained with sodium phosphotungstate except (f) which was stained with ammonium molybdate.

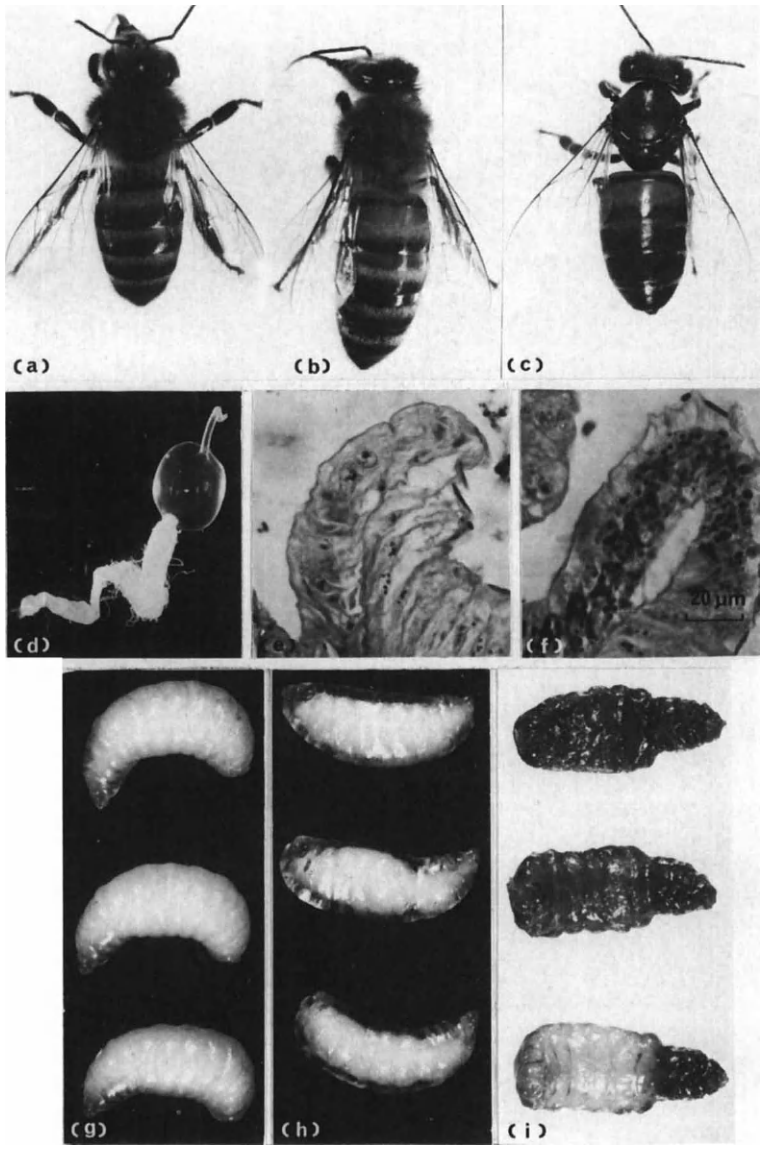

Figure 36 Chronic bee-paralysis: (a) healthy individual; (b) Type 1 syndrome; (c) Type 2 syndrome; (d) gut of bee with Type 1 syndrome; the bloated honey-sac causes distension of the abdomen (see (b)); (e,f) longitudinal sections of gut epithelium immediately posterior to the Malpighian tubules of a healthy bee and of a bee with chronic paralysis, showing Morison's cell inclusions in the latter (Heidenhain's iron haematoxylin).
Sacbrood: (g) healthy individuals; (h) early stage of disease; (i) formation of scale (bottom to top).

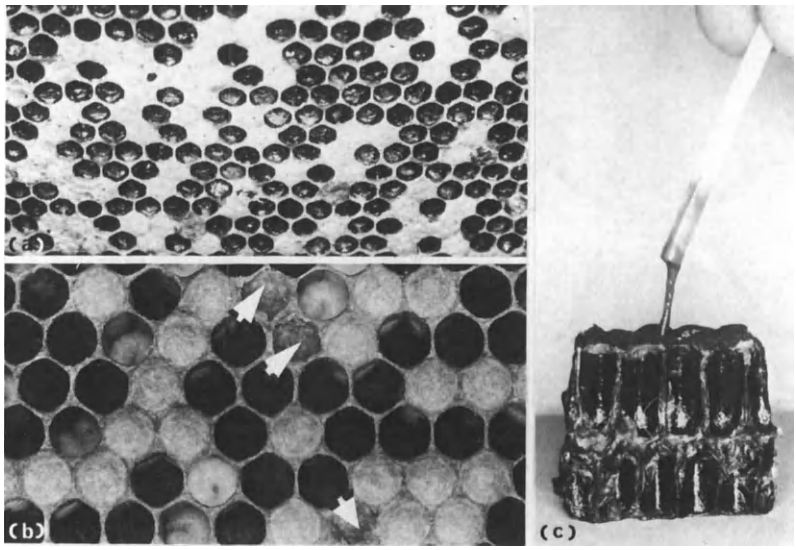

Figure 37 American foulbrood: (a) oblique view from above of comb with remains ("scales") of severely attacked brood; (b) dark cappings (arrowed) of dead pupae among healthy brood; (c) ropy thread formed with larval remains.

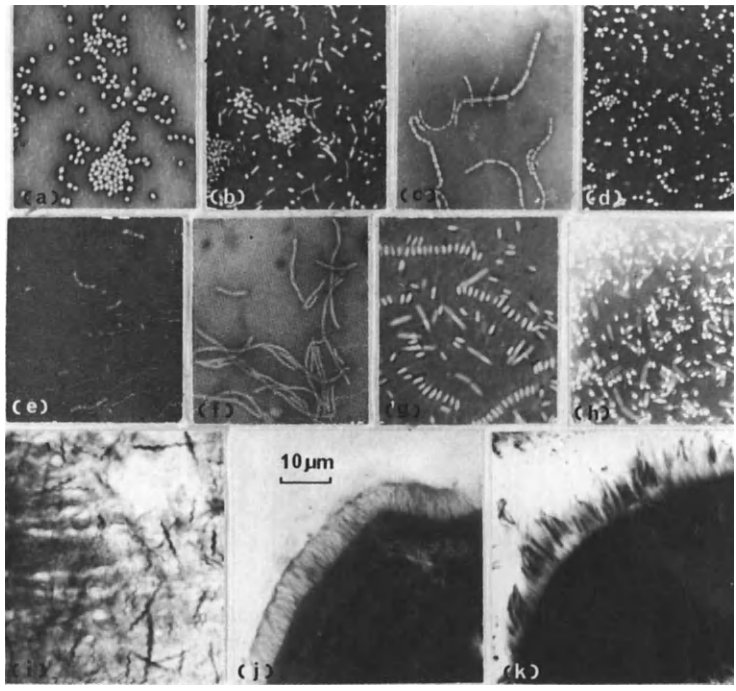

Figure 38 Bacteria of European and American foulbroods (× 650): (a) *Melissococcus pluton* from larvae; (b) *Melissococcus pluton* + "Bacterium eurydice" from larvae; (c) *Melissococcus pluton* from culture; (d) *Streptococcus faecalis* from culture; (e,f) "Bacterium eurydice": coccal and rod forms of one strain cultivated on pollen-extract and honey-based media respectively; (g) *Bacillus alvei* spores; (h) *Bacillus larvae*: sporulating culture; (i) *Bacillus larvae*: coalesced flagella in gut contents of a young larva; (j) Brush border of the mid-gut cells of a healthy larva; (k) *Bacillus larvae*: vegetative cells in the brush border of the mid-gut cells of a young larva.

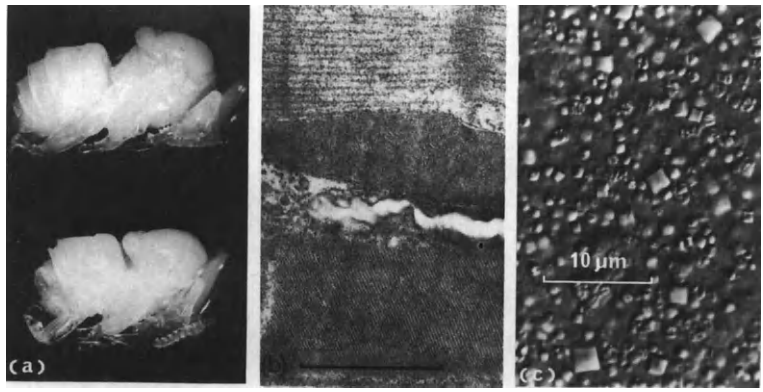

Figure 39 (a) Healthy pupa (top) and a pupa from a larva that had been infected with *Melissococcus pluton*; (b) ultrathin section of thoracic muscle of an adult bee infected with cloudy-wing virus showing (top to bottom) muscle fibril, sarcosome, tracheole, crystal of particles; (scale bar; 1 µm) (c) cubic bodies of a polyhedrosis virus that attacks wax-moths.

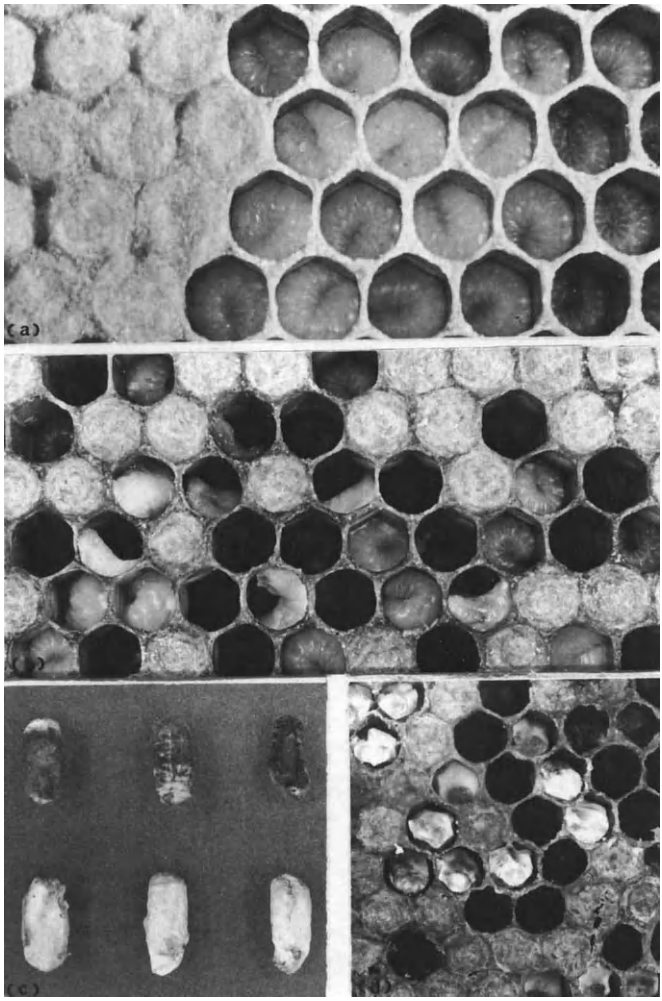

Figure 40 European foulbrood: (a) healthy larvae, capped larvae on the left; (b) larvae with European foulbrood. **Chalk-brood**: (c) top row: prepupae covered with fruiting bodies of *Ascosphaera apis*; bottom row: prepupae killed by a single strain of *A. apis*; (d) severely attacked brood.

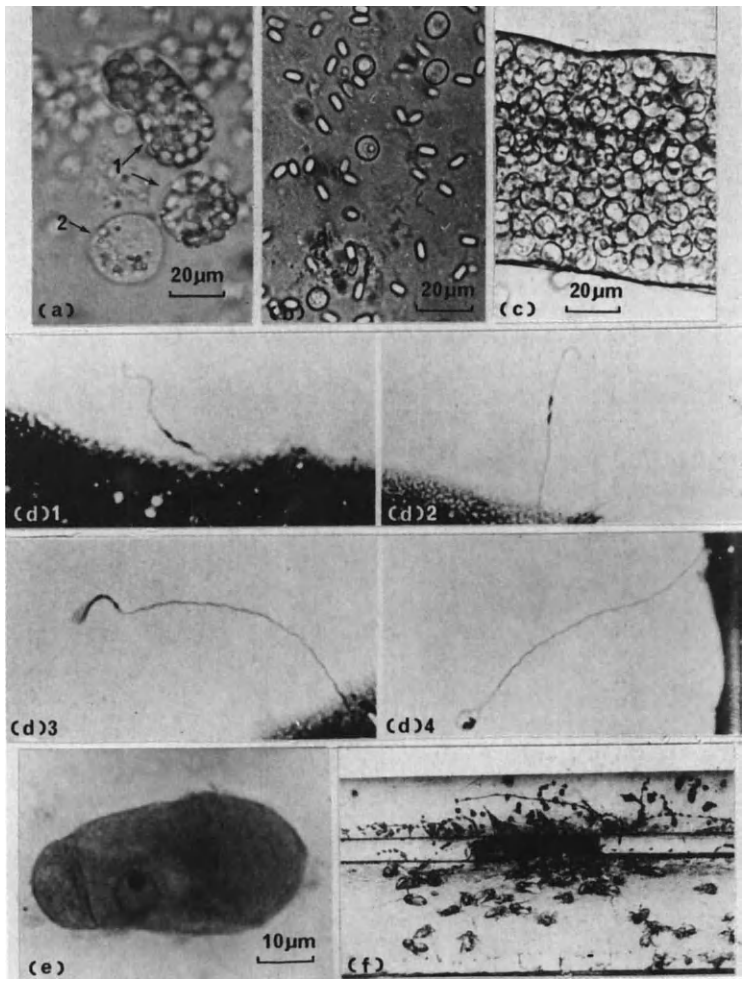

Figure 41 (a) *Nosema apis*. Live epithelial cells of the mid-gut cast off into the gut contents of infected bees, 1. cells packed with spores, 2. uninfected cell; (b) spores of *N. apis* and cysts of *Malpighamoeba mellificae*; (c) cysts of *Malpighamoeba mellificae* in a section of the Malpighian tubule of an adult bee; (d) filaments of *N. apis* extruding from hanging drops, showing the passage of the twin nuclei down the hollow filament (1, 2) and their emergence in the sporoplasm (3, 4), which in nature is injected into the host cell (Courtesy of J. P. Kramer); (e) gregarine from the mid-gut of an adult bee (Courtesy of J.D. Hitchcock); (f) "Dysentery": photograph taken in 1911 of entrance of a bee colony alleged to have "Isle of Wight disease"; appearance of dead bees at hive entrance and of faecal matter on hive parts are not uncommon after long winter confinement of normal bee colonies. (Courtesy of the Bee Research Association.)

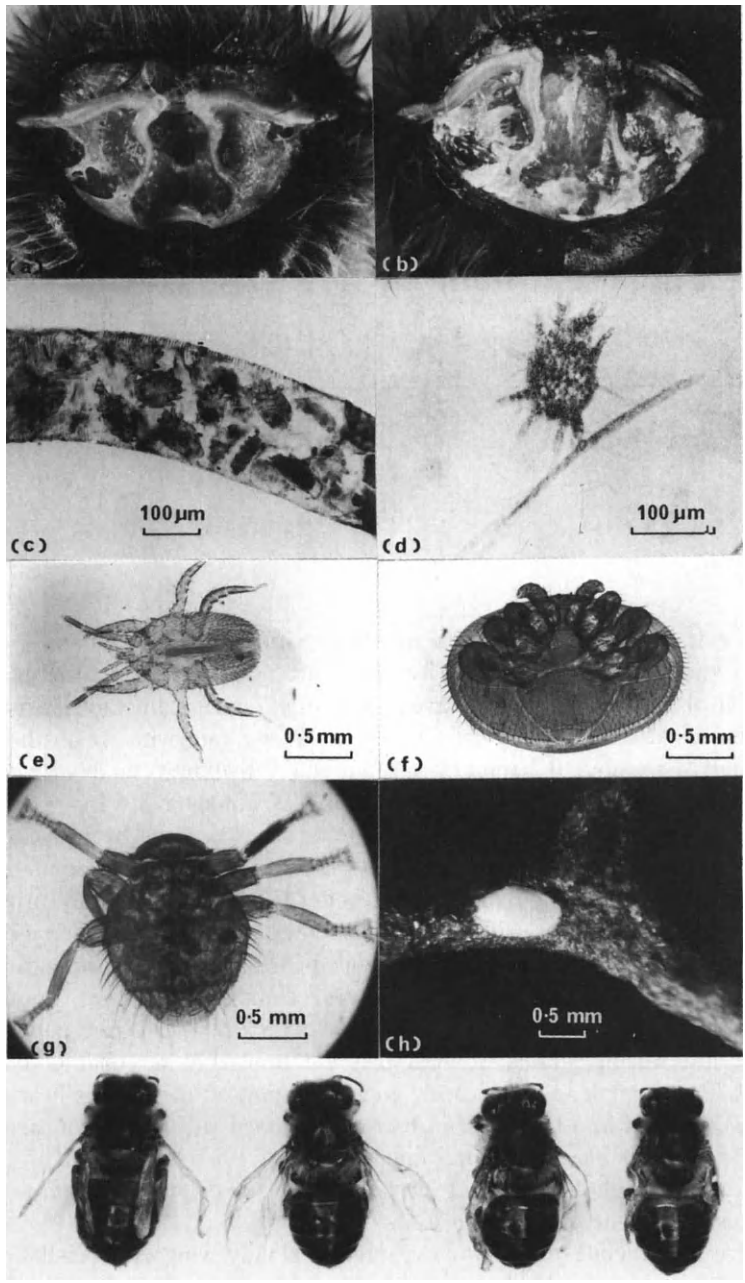

Figure 42 Parasitic mites, and *Braula* coeca; (a) anterior view of thoracic tracheae of healthy adult bee after removal of head and first thoracic segment; (b) tracheae blackened unilaterally as a result of infestation with *Acarapis woodi*; (c) trachea filled with individuals of *A. woodi*; (d) Migrating female of *A. woodi* gripping a hair of its old host with one leg and ready to attach itself to the hair of a passing new host; (e) *Tropilaelaps clareae*; (f) *Varroa jacobsoni* (female); (g) adult *Braula* coeca; (h) egg of *Braula* coeca on the edge of a honeycomb cell ((a, b and c) courtesy of HMSO); (i) bees killed by deformed wing virus transmitted by *Varroa jacobsoni*.

10
The Treatment of Bee Diseases

I. VIRUSES

At present, and in common with nearly all virus diseases of animals, there are no known direct treatments for virus infections of bees. However, it is probable that black queen-cell virus, bee virus Y and filamentous virus will be suppressed indirectly by the control of *Nosema apis*, with which they are intimately associated (Chapter 3, V.). Measures that prevent dysentery will also probably prevent the spread of bee virus X (Chapter 3, VI.).

Beekeepers have long believed that paralysis can be cured by replacing the queen of an affected colony with one from elsewhere. There is much circumstantial evidence to support this belief (Chapter 3, I.B.) and a policy of replacing old queens with some from other parts of the country rather than allowing colonies constantly to requeen themselves and become inbred, is probably a good precautionary measure. On the other hand, importing queens from great distances runs the risk of bringing in exotic pathogens, and of introducing strains of bees that are unusually susceptible to local viruses. For example, a significantly greater proportion of colonies headed by queens imported into Britain from Russia developed visible signs of sacbrood than did colonies headed by local queens (Bailey, 1967c). A degree of cross-breeding that would be likely between well dispersed colonies in nature would probably be the ideal compromise.

There is evidence that chronic paralysis, cloudy wing and Kashmir bee viruses are transmitted most readily either by direct contact or by aerial transmission between individuals when these become unusually crowded. Beekeeping methods that inhibit foraging or otherwise lead to crowding and confinement can be expected to exacerbate these, and possibly other, virus infections. Keeping hives on stands, with their entrances well clear of the ground to prevent diseased individuals from crawling back, may be helpful.

II. BACTERIA

A. *American Foulbrood*

1. Destruction of diseased colonies

This is best when the incidence of disease is low. The colony is killed with about half a pint of petrol poured in the top of the closed hive, and then burned in a shallow pit which is afterwards filled in. The boxes and equipment are usually flamed thoroughly with a blow torch, but a better treatment is to immerse them for 10 min in paraffin wax heated to 150°C (Cook, 1968). There is no doubt that destruction suppresses the incidence of American foulbrood; but the evidence suggests that it cannot eradicate endemic disease from large areas. For example, a compulsory scheme of destruction that operated in Switzerland from 1908 was followed by a fall in the incidence of American foulbrood from approximately 13 to 2% after 25 years (Leuenberger, 1933). Furthermore, the number of colonies inspected increased over the years and the actual number of those found diseased fell only from about 90 to about 40 or 50 per year at the end of the period. In England and Wales, the number of colonies found and destroyed each year for 20 years since 1954 did not fall appreciably from an average of about 800 out of 80 000 colonies inspected each year (Anon., 1947–1980).

2. Manipulative treatment

Shaking adult bees from diseased colonies onto new combs has often been tried, but the method is unreliable. In one investigation (Winter, 1950) disease incidence could not be decreased below 2%; in another, 81 colonies out of 300 that were treated redeveloped disease in the following 2 years (Dunham and King, 1934); and many colonies derived from adult bees that were shaken into empty boxes from diseased colonies, fed syrup for 72 h and subsequently transferred to wax comb-foundation became diseased again (Sturtevant, 1933). The method was officially endorsed in Denmark but, after many years, American foulbrood remained the commonest honey bee disease in the country (Kaare, 1952, 1953).

Improved methods of transferring bees have been suggested. Bees can be anaesthetized with smoke from fuel impregnated with potassium nitrate. They then fall off the combs without gorging themselves on honey as they do when they are smoked in the usual way. This method, followed by no feeding for about 24 h, considerably decreases the chance of spores in honey being transferred to the new combs; but it is laborious, and the old combs which have to be destroyed are at least as valuable as the bees.

3. Sterilization of combs and honey

Much effort was devoted to methods of sterilizing combs and equipment in the 1920s and 1930s. Formaldehyde was most frequently used; it was evaporated from its solution (formalin) at normal temperatures, by heat, or by adding potassium permanganate or barium peroxide to formalin in closed boxes containing the combs; alternatively, the combs were soaked in soap–formalin, alcohol–formalin or glycerol–formalin mixtures. All the methods work to a large extent. The simplest is fumigation with vapour from excess formalin at room temperature, and it seems no less efficient than the others. Combs enclosed with about 150 ml of formalin per 25 litres of volume at normal temperatures are mostly completely sterilized after 14 to 17 days; open cells are sterile after 6 days. The main drawback is the difficulty of sterilizing capped cells and the permanent toxicity to bees of honey and pollen in the fumigated combs.

Chlorine, either as a gas or in watery solution, is effective, but it makes combs brittle and severely corrodes metal parts (Hitchcock, 1936). It seems to be no more efficient than formalin.

More recently, interest has revived in methods of sterilizing combs, because their value and their importance in the transmission of disease, together with the shortcomings of chemotherapy, have become better appreciated. Shimanuki *et al.* (1970) showed that boxes of combs from colonies with American foulbrood were almost completely sterilized after humidification for 24 h and then treated with ethylene oxide at 1 g/litre for 48 h at 43°C in fumigation chambers. Boxes can be fumigated cheaply, and possibly effectively, in plastic bags filled with a mixture of ethylene oxide (10%) and carbon dioxide for about a week at 27°C (Winston, 1970); but for best results, heated, humidified, gas-tight containers are required (Gochnauer and Corner, 1976). However, ethylene oxide forms an explosive mixture with air and its use, even in gas-tight chambers, does not guarantee sterility (Gochnauer *et al.*, 1979). Furthermore, it may leave carcinogenic residues in foodstuffs and is banned for this reason in some countries.

The fumigation of empty comb and equipment that have been in contact with American foulbrood is probably the safest and most economical practice. Combs containing dead brood are best destroyed by burning.

Although honey can be sterilized by acidification with phosphoric acid followed by heating (White and Sturtevant, 1954) the process makes honey extremely poisonous for bees (Chapter 9, I.).

Combs can be disinfected with gamma irradiation from Cobalt-60. Gochnauer and Hamilton (1970) found that, although absolute sterility is not readily achieved by 10 kilograys (= 1 M rad), infectivity was almost completely eliminated by 2 kilograys. Such a process could be economical when done

on a sufficiently large scale using commercially available facilities.

Many thousands of hive-boxes of naturally contaminated combs that were irradiated in Australia with 10 kilograys did not subsequently transmit American foulbrood, *Nosema apis* or wax-moths, although they still transmitted some European foulbrood (Hornitzky, 1986). The performances of colonies established on similarly irradiated combs were unimpaired when compared with untreated healthy colonies (Ratnieks, 1987). Irradiation with high-velocity electron beams, normally used for the industrial sterilization of medical products, will also sterilize combs contaminated with *Bacillus larvae* and the cost compares favourably with that for fumigation with ethylene oxide (Shimanuki *et al.*, 1984).

4. Chemotherapy

Sodium sulphathiazole effectively suppresses American foulbrood when 0.5–1.5 g are given in 5–15 litres of strong sucrose syrup to a diseased colony (Haseman and Childers, 1944). The drug is stable in honey for several years and can be supplied in autumn to act successfully during the following year (Katznelson and Jamieson, 1955).

Oxytetracycline ("Terramycin") is also effective when 0.25–0.4 grams in 5 litres of syrup is supplied to a diseased colony. All its activity disappears after about 2 months in honey but higher concentrations are toxic for bees (Section B.4.).

Other methods of applying drugs have been recommended, principally dusting them over the combs in dry sugar, but at best this is no more effective than the methods described and is more likely to damage brood. Mixing drugs with vegetable fat and sugar, and applying them in so-called "extender-patties" may be more efficient than applying them in syrup in some circumstances (Wilson *et al.*, 1971a).

The efficiency of drug treatment varies widely. The degree of contamination of equipment, the ability of the beekeeper and the variability of the many natural factors that influence the course of disease all affect the issue.

Chemotherapy has no effect on spores that contaminate combs and equipment. Its use can lead to the spread of such infection between colonies and so to an increasing dependence on regular application. Accordingly, chemotherapy is not advisable when the incidence of American foulbrood is low and can be contained readily and economically by the destruction of relatively few colonies. Nevertheless, when applied efficiently, chemotherapy can decrease infection within diseased colonies, sometimes with remarkably little recurrence of disease (Wilson *et al.*, 1971b). Chemotherapy becomes economically attractive when disease is widespread, but it would be advisable to withhold treatment after one or two seasons of use to see whether it is needed any longer.

5. Resistant strains of bees

A certain degree of resistance to American foulbrood is common in bees (Chapter 4, I.D.), and tests in the USA by Park (1935) showed that some colonies were more resistant to American foulbrood than others. The early tests were done with an inoculum per colony of the dried remains of 75 larvae killed by the disease, which is about the critical amount (Chapter 4, I.D.): the resistant colonies were unable to overcome bigger inocula (Eckert, 1941; Filmer, 1943; Woodrow, 1941a, 1941b, 1942). Woodrow and Holst (1942) noticed that many individually infected larvae in the resistant colonies were removed about 6 days after they were sealed in their cells, whereas very few sister larvae placed in control colonies had been removed 11 days after they were sealed, by which time they were full of infective spores. This was confirmed by Rothenbuhler (1958) and his colleagues who made further selections for resistance and susceptibility, increasing both about ten-fold compared with the average.

Rothenbuhler and his associates went on to demonstrate a range of hereditary factors that contributed towards resistance. These comprised

1. The efficiency of the hygienic behaviour of adults in removing diseased larvae, which was further separable into a factor for uncapping the cells and a factor for removing the larvae (Rothenbuhler, 1964);
2. The rate at which young larvae became innately resistant to infection with increasing age (Bamrick and Rothenbuhler, 1961);
3. The efficiency of adults in filtering the spores of *B. larvae* from food by means of their proventriculus and/or the efficiency of a bactericidal factor in the gland secretions of nurse bees (Thompson and Rothenbuhler, 1957).

The most striking and unexpected fact about hygienic behaviour of the susceptible and resistant strains of bees selected by Rothenbuhler, was that the genes which determined both the prompt uncapping of cells and the efficient removal of the larvae in them were recessive. Hybrids of the two strains were all susceptible, and when these were cross-bred with the parent strains a quarter of the offspring were susceptible, a quarter were resistant, a quarter would uncap cells but did not remove the larvae, and a quarter would remove the larvae only when the cells were uncapped for them. This is explicable on the hypothesis that uncapping cells and removing larvae each depend for full expression upon homozygosity for two independent recessive genes. This unfortunately entails inbreeding, which, apart from its practical difficulties, can have undesirable consequences (Chapter 3, I.B.; Chapter 9, IV.).

The rate at which larvae of the resistant strains selected by Rothenbuhler

became innately resistant to infection (Chapter 4, I.C.) was greater than that of other strains, possibly because they grew quicker during their first day of life (Sutter *et al.*, 1968) and because their food contained more factors that inhibited the germination of spores of *Bacillus larvae* and the growth of vegetative cells (Rose and Briggs, 1969). Also the adult bees of the resistant strain filtered spores from the honey in their honey-sacs more efficiently than susceptible bees (Plurad and Hartman, 1965). Nothing is known of the genetic basis of these aspects of resistance, but Hoage and Peters (1969) were able to select for even greater resistance from the resistant strains by inoculating young larvae, especially drones, with a critical number, about 1000, of spores of *Bacillus larvae* and in-breeding from the survivors by artificial insemination.

The protective ability of adult bees either to filter off bacterial spores or to decrease their infectivity with bactericidal agents, or to do both, was also demonstrated in bees from Hawaii. American foulbrood was rampant in Hawaii during the early 1930s but was less evident in 1949 (Keck, 1949). It was thought that very resistant bees had survived the virtual abandonment of beekeeping in Hawaii during World War II, although 13 out of 86 colonies were found diseased when examined in 1949, which is still a high incidence. However, Eckert (1950) noted that the colonies stored a great deal of honey and that those found diseased before the nectar-flow became apparently healthy afterwards. Hundreds of colonies were then examined in abandoned apiaries, where combs had been largely destroyed by wax moths; and numerous swarms in cliffs and trees were also examined, but no disease was found. Resistance seemed likely, and Thompson and Rothenbuhler (1957) showed that bees from Hawaii protected larvae from American foulbrood about eight times more efficiently than colonies that had been selected for susceptibility were able to protect sister larvae. The diminished population of colonies in Hawaii after World War II probably decreased the rate at which American foulbrood had spread previously, and they probably fared much better in good years than they did before, thus being more able to ward off disease (Chapter 4, I.D.), but the increased hereditary ability of their bees to protect larvae may have been an important factor contributing towards their resistance.

There is no doubt that there are several inheritable factors that make some strains of bees more resistant than others to American foulbrood. The differences found between resistant bees and the average strains of bee are not sufficient to sustain hopes of eventually being able to select immune strains; and the task of separating the desirable from the undesirable characters, combining them and maintaining them, would be difficult in present circumstances. Nevertheless, the work of Rothenbuhler provides the best scientific evidence of hereditable resistance to disease in insects and is a solid foundation of knowledge about disease resistance in bees.

6. The effects of beekeeping

Infection by *Bacillus larvae* is the most unstable of all infections in honey bee communities. The balance is easily tipped in favour of its spread and it can be expected to increase once established in beekeepers' colonies, which have less opportunity to express the little resistance they have than undisturbed colonies. In view of the longevity of the spores of *B. larvae*, untreated equipment from diseased colonies should always be considered infective.

B. *European Foulbrood*

1. Destruction of diseased colonies

This has been required by law in some countries and, although it may help to decrease the incidence of the disease, it has not been very effective and is almost certainly uneconomical. Destruction of colonies with either American or European foulbrood in Switzerland from 1908 to 1933 and in Britain from 1942 to 1967 did not decrease the incidence of European foulbrood, whereas the incidence of American foulbrood in Britain declined from about 7% of colonies to about 1% by 1954 (Anon., 1947–1980). However, it is probably best to destroy severely diseased colonies, especially when the incidence of disease is low.

2. Manipulative treatments

Many have claimed that European foulbrood can be eradicated by removing the queens of diseased colonies for between 3 days and 3 weeks and then replacing them with newly mated queens (Langstroth, 1866; Phillips, 1921; Sturtevant, 1920). The idea is to give bees an opportunity to clear away diseased larvae and bacterial contamination. However, most diseased colonies recover anyway (Chapter 4, II.C.) and the queenless period almost certainly impairs the efficiency of this process. Dzierzon (1882) noticed that the larvae in the newly formed queen cells of dequeened colonies were frequently killed by European foulbrood. Such colonies retain relatively more infected larvae than normal colonies, whereas those with prolific young queens soon have many young larvae to feed and consequently eject more infected ones than usual (Chapter 4, II.C.). Requeening diseased colonies helps the process of spontaneous recovery but a queenless period is almost certainly valueless, and may be harmful.

3. Sterilization of combs and honey

Fumes of formaldehyde or acetic acid, applied in the same way that is advised for the disinfection of comb contaminated with spores of *Nosema apis* (Section IV.A.1.), will kill the resting stages of *Melissococcus pluton* when these are not buried beneath organic matter. Sound, empty comb from diseased colonies are worth treating in this simple fashion. Comb containing dead brood should be burned.

Ethylene oxide, applied as for disinfecting comb from colonies with American foulbrood (Section II.A.3.) will probably be at least equally effective for European foulbrood. In contrast with American foulbrood, gamma irradiation with up to 8 kilograys was ineffective (Pankiw *et al.*, 1970), although 10 kilograys significantly decreased the infectivity of naturally contaminated combs (Hornitzky, 1986).

4. Chemotherapy

Several different antibiotics effectively suppress European foulbrood, but the disease sometimes recurs in treated colonies especially during the following season. Although recovery from the disease is accelerated by antibiotics, infection and contamination by *Melissococcus pluton* may not decrease as much as in colonies that are allowed to recover spontaneously. Many infected larvae, that would usually be ejected by adult bees, survive when treated with antibiotics, but they still leave many infective bacteria in their faeces when they pupate. For best effect, antibiotics should be administered before the time when European foulbrood is usually seen, as early in the season as is practicable. Then the multiplication and accumulation of *Melissococcus pluton* is forestalled, disease outbreaks are decreased in severity, or do not occur, and residual infection is readily controlled by the usual activities of the bees (Chapter 4, II.C.).

A simple method is to give 0.5 or 1 g, to small or large colonies respectively, of oxytetracycline ("Terramycin") dissolved in about 500 ml of concentrated sucrose syrup, by sprinkling the syrup either over the bee cluster in the hive in warm weather, or into unoccupied comb taken from the edge of the colony in cold weather. These combs are replaced with the syrup side outwards from tbe bees to prevent them from consuming it too quickly, and to allow it to become mixed gradually with other food that is in the colony or given subsequently. This is necessary because the antibiotic is toxic for bees and brood in the concentration described (Gochnauer, 1954). It is still effective but harmless when more dilute, e.g. 1 g in 5–10 litres of syrup, but it is often impracticable to give such large volumes in early spring. Colonies that are too severely diseased to recover quickly should be destroyed as for American foulbrood (Section II.A.I.) Oxytetracycline decomposes in honey, which is

safe for human consumption 8 or more weeks after treatment (Landerkin and Katznelson, 1957).

Other antibiotics are equally effective, but they should not be used, either because they could be harmful for anyone consuming honey contaminated with them, or because those that are harmless are best held in reserve in the event of resistance developing towards oxytetracycline.

Other methods of application, such as dusting dry powders containing antibiotics or feeding several small doses at frequent intervals have been advocated, but none have been shown to be more effective than the method described above.

5. The effects of beekeeping

Methods of beekeeping that suppress brood-rearing or maintain a small amount of brood in proportion to nurse bees, allow *M. pluton* to multiply and spread because they cause a temporary excess of glandular food supplied by nurse bees to larvae (Chapter 4, II.C.). For this reason disease frequently becomes severe in colonies that are regularly used for producing queen-cells or royal jelly.

In localities with uninterrupted nectar-flows, where colonies can grow unhindered each year, infection often remains slight and disease inapparent. However, when colonies are moved their development is checked. European foulbrood often becomes apparent after they have settled down in their new site and have begun to grow again. The disease often breaks out in endemically infected colonies after they have been brought back from the pollination of orchards in the spring.

III. FUNGI

A. *Chalk-brood*

Chalk-brood is greatly aggravated by practices that cause the loss of heat from bee colonies, especially in spring and early summer when the natural incidence of chalk-brood is highest. Procedures that cause the loss of heat or that do not allow bees to maintain adequate temperatures have been used successfully to exacerbate chalk-brood in colonies for experimental purposes. For example, placing many combs of sealed brood from slightly diseased colonies into weak colonies, or removing many of the adult bees of infected colonies, or giving them extra brood to rear, have effectively increased the amount of disease (De Jong, 1976). The most usual equivalent practices in beekeeping, that cause trouble, are the methods employed to prevent swarming,

which involve forcing the colony to expand either by dividing it or by spreading it into a larger space (Cale *et al.*, 1975). The colonies of beekeepers who do this routinely, or too early in the year, frequently suffer from chalk-brood. This is resolved when infected colonies are left undisturbed or when swarm control methods are delayed as long as possible. Chalk-brood recurs in such colonies, because the spores of *Ascosphaera apis* remain infective for many years, but its severity is greatly diminished.

Heath (1982a) lists more than 30 chemicals that have seemed promising for controlling infection, or that at least inhibit *A. apis* in culture; and Glinski (1986) reported that the choline salt of *N*-glucosylpolyfungin effectively controlled chalk-brood when 100 mg in 250 ml syrup were sprayed on colonies four times at 5 day intervals, seemingly suppressing it completely with a further course of treatment the following season. However, the persistence of spores would make eradication of the disease by such treatments unlikely.

Spores in the larval remains are more easily killed by ethylene oxide fumigation than the spores of *Bacillus larvae* (Section II.A.3.) (Gochnauer and Margetts, 1980).

B. *Other Fungi*

No successful treatments are known.

IV. MICROSPORA AND PROTOZOA

A. Nosema apis

1. Manipulative treatment and comb sterilization

Although it is theoretically possible to remove infection by transferring colonies to uncontaminated combs (Chapter 6, I.C.) in early summer, the method is costly in labour at least, and there are many factors, some of which have already been discussed, which can cause re-contamination. Moving colonies entirely on to new wax foundation in the autumn and feeding sufficient syrup for them build new comb and survive the winter has proved more successful, probably because re-contamination of comb is then less likely than in spring (Fries, 1988), but the cost is still high. In most circumstances it is probably cheaper to avoid aggravating infection rather than to try to eradicate it, especially as enzootic infection is so common.

Combs that contain no honey or pollen can be disinfected with formalin, but when there is food in the combs formalin makes it extremely poisonous

for bees: then the most convenient safe fumigant is acetic acid (Bailey, 1955b; Hirschfelder, 1957; Jordan, 1957; Lunder, 1957). Commercial grades of either fluid may be used; acetic acid is available in a concentration of 80% which stays liquid below 15°C, unlike 100% or "glacial" acid. The easiest effective method of using these fumigants is to intersperse absorbent materials between piles of hive bodies containing the combs, and to pour about 150 ml of the liquid on to the material between each box. The stacks should be left in a warm corner out of doors and protected from direct winds for a few days before being used. It is best to air them for a day before putting them in colonies.

Keeping contaminated combs at 49°C and 50% relative humidity for 24 h greatly decreases their infectivity without damaging them (Cantwell and Lehnert, 1968; Cantwell and Shimanuki, 1970).

2. Chemotherapy

The only successful drug found so far is the antibiotic fumagillin, which is derived from *Aspergillus fumigatus* and marketed especially for beekeepers. It quickly suppresses infection when fed to bees in concentrations between 0.5 and 3 mg/100 ml syrup, and there are no known ill-effects (Katznelson and Jamieson, 1952a). The activity of the antibiotic remains high in honey kept at 4°C for several years (Furgala and Gochnauer, 1969a), and for at least 30 days at 30°C (Furgala and Sugden, 1985). When about 200 mg of fumagillin in 4.5 or 9 litres of syrup is fed to each colony in autumn, infection is markedly decreased the next spring (Bailey, 1955c). Up to 400 mg per colony is recommended when infection is known to be severe, especially when winters are long, as in northern Canada (Tibor and Heikel, 1987). When fed in spring, fumagillin prevents the usual peak of infection and treated colonies produce significantly more brood and honey than similar colonies fed syrup only (Farrar, 1954; Furgala and Gochnauer, 1969b; Gochnauer, 1953; Jamieson, 1955; Moeller, 1962). Impressive increases of honey yields, brood, and life-span of adult bees were obtained by Woyke (1984) when he fed 50 to 100 mg fumagillin, distributed in a few small volumes (e.g. 250 ml × 5) of syrup, to each colony during a week or two before the main nectar flow. Spring feeding may not have such a long-term effect as autumn feeding because spores, which are mostly deposited on combs in late winter (Chapter 6, I.C.), are unaffected by the antibiotic. Some investigators have found fumagillin ineffective when applied as a dust or in solid sugar candy (Furgala and Gochnauer, 1969b), but others found it satisfactory when fed in sugar candy in autumn (Szabo and Heikel, 1987) or when supplied in dry powdered sugar (Wyborn and McCutcheon, 1987) or in pollen supplements in spring (Moeller, 1978). Some variability of success, particularly of treatment in

spring, may depend on the degree of contamination on combs, which will re-infect bees as colonies expand and when their supply of antibiotic becomes exhausted.

Attempts to select strains of *N. apis* resistant to fumagillin, by maintaining infection in bees treated with subliminal doses of the antibiotic, have failed (Gross and Ruttner, 1970).

3. The effect of beekeeping methods

There are several ways in which beekeeping practices aggravate infection. Contaminated combs placed in colonies towards the end of summer, as they often are during late nectar-flows, will reintroduce infection (Fig. 19), too late for the bees to clear it up adequately by autumn. Severely infected clusters will then dwindle more rapidly than usual during the winter or may even die.

Individual bees are frequently crushed when colonies are opened and examined, and they are then removed by the other bees which ingest the liquid remains. This aggravates any infection and probably explains the high incidence of *Nosema apis* in "package bees" (Chapter 6, I.D.). These are small clusters or colonies of bees produced in warm regions, such as southern USA, for sale to beekeepers in northern areas where it is more expensive to keep colonies throughout the winter than to destroy them in autumn, take all the honey and buy more bees the following spring. The constant handling suffered by the parent colonies, followed by the confinement of the bees in the package, maintain severe infections (Farrar, 1947; Reinhardt, 1942).

Infection is aggravated by transporting colonies to new sites where there may be desirable nectar-flows or where pollination is required (Bailey, 1955b). The bees often end such journeys in a distressed condition, and many then deposit faecal matter within the colony, even on the cappings of newly sealed honey.

The most dangerous time to disturb colonies is during late winter and early spring when the bees have been confined for long periods (Table X). In practice, infection is most likely to be spread by moving or disturbing colonies during the spring, because spontaneous infection of bees has become most severe at this time when they are most actively cleaning contamination from the combs. The practice of taking colonies to fruit orchards for spring pollination has considerably increased in recent years and probably causes the severe infections suffered by colonies of many beekeepers engaged in this work. Many beekeepers also frequently examine their colonies too soon in spring and early summer.

Commercial beekeepers who are compelled to move and handle colonies a great deal may find it advantageous to practise a rotation system, keeping

some hives undisturbed at good sites for a year and allowing the natural control of infection to take full effect.

Queen rearing is another beekeeping practice likely to aggravate infection. The small colonies of bees used to keep and mate virgin queens ("mating nuclei") are populated by bees that are older than average and so more likely to be infected. This is because little brood is produced by the newly mated queens before they are replaced with another to be mated, and the colonies are also frequently handled (Table X).

The same effects caused by poor seasons on infection are brought about by keeping too many colonies together, which then cannot support themselves on the available forage. This may largely explain the high proportion of infection in urban regions, for instance the 30% of colonies in the urban south-east of England compared with 3–16% in the other areas of England and Wales (Anon., 1947–1980), and 30–90% of colonies infected in experimental stations in England, Scotland and Russia (Morison *et al.*, 1956). These high incidences of infection are also aggravated by the unusual amount of handling suffered by many of the colonies.

Table X The number of bee colonies found infected with *Nosema apis* at the end of April in two successive years at Rothamsted.

Group	Treatment	Year			
		1		2	
		Infected	Healthy	Infected*	Healthy
1	Colonies transferred to disinfected combs during early summer of Year 1 and increased by division	12	0	5	18
2	Combs examined monthly for other tests during winter of Year 1/Year 2	3	11	10	0
3	Colonies without queens for several weeks during summer of Year 1	4	11	8	2
4	None	79	111	71	109

* The proportion of infected colonies in Year 2 changed significantly from that in Year 1 in all groups except 4, showing that: (Group 1) transferring colonies to disinfected combs removes much residual infection; (Group 2) disturbing colonies aggravates infection and (Group 3) colonies without queens become more infected than usual (original data).

B. Malpighamoeba mellificae

1. Manipulative treatment and comb sterilization

Infection can be removed by transferring colonies in early summer to non-contaminated combs (Fig. 20), a procedure less likely to fail with *M. mellificae* than with *Nosema apis* (Chapter 6, II.C.). However, it is probably more economical to suppress rather than to try to eliminate infection. This can be done by fumigating spare combs in the same way as for *Nosema apis* (Section IV.A.1.).

2. Chemotherapy

Few attempts at chemotherapy have been made and none have proved effective (Bailey, 1955d; Giordani, 1959).

3. The effect of beekeeping methods on infection

The equivalent section for *Nosema apis* (Section IV.A.) applies equally to *M. mellificae*. In Britain, most infection (3% of colonies) is in the south-east near London, probably for the same reasons as for *Nosema apis*. In Denmark too, most infection by *M. mellificae* has been found near towns, particularly near Copenhagen (Fredskild, 1955). Nevertheless, infection by *M. mellificae* remains low compared with that by *Nosema apis*, probably because cysts develop slowly, maturing only when bees are nearing the end of their normal summer lives, so there is far less likelihood of combs becoming contaminated with them in summer than with spores of *N. apis* (Chapter 6, II.B.).

V. PARASITIC MITES

A. Acarapis woodi

1. Hygienic methods

If a brood is not infested it may be separated, when sealed, from infested colonies and used to create new uninfested ones by hatching it in incubators and providing a new uninfested queen; or it may be added to uninfested colonies. This idea, with added complications believed to increase efficiency, has been advocated many times. The principle has been tested on a large scale in Europe and has apparently completely eradicated the mites (Kaeser, 1952). The method is laborious, however, and involves the loss of bees and queens.

2. Chemotherapy

A wide variety of chemicals will selectively kill mites in bee colonies (International Bee Research Association, 1986). They range from simple materials, such as sulphur, to complex compounds of unknown composition. Many, perhaps most, are harmful for bees to some degree. Almost all are applied as vapours.

Probably any non-specific toxic vapour will kill mites sooner than bees because the surface area of an individual of *A. woodi*, relative to its volume, is about 100 times that of a bee. Accordingly, a lethal dose will accumulate more quickly in mites than in bees provided they are about equally permeable. This differential effect can, no doubt, be optimized with suitable concentrations of moderately toxic vapours applied for limited periods in favourable temperatures. However, the necessary conditions are likely to be limited in range and difficult to quantify and manage. This may well explain the varied results obtained with the same materials by different investigators, or at different times. The spontaneous recovery of infested colonies can also be very misleading in inadequately controlled tests (Wille *et al.*, 1987).

Relatively specific, usually synthetic acaricides are available. Some are best applied as a smoke from smouldering paper strips impregnated with the compound; others volatilize spontaneously from impregnated strips of wood or plastic; and some can be applied in aqueous solution as aerosol sprays. A few are systemic and can be fed in syrup to bees. None are without disadvantages and need limited ranges of conditions for best effect. Some, perhaps all, damage bees to an extent, at least because of the way they have to be applied. Some of these materials leave, or are suspected to leave unacceptable, persistent residues in honey, which inclines modern attitudes towards the use of more familiar, but less specific materials that are believed to be comparatively safe for man. These include the vapours of menthol, tested and found effective in laboratory tests by Vecchi and Giordani (1968), and since found to be effective in bee colonies (Cox *et al.*, 1989a; Herbert *et al.*, 1987), and of formic acid (Bracey and Fischer, 1989; Hoppe *et al.*, 1989). Both are repellant and injurious to bees.

Menthol is usually applied as 25 g of crystals or cakes placed on the top of the combs of a colony for up to one or two months; and formic acid can be applied as a liquid (70% industrial formic acid) soaked on absorbent cardboard sheets, about 30 × 20 × 0.15 cm, placed on the floor of the hive away from the entrance. The period of treatment and number of applications for the best effect are probably influenced mostly by the ambient temperature. Two treatments at 7 day intervals were effective with formic acid in Pakistan (Hoppe *et al.*, 1989).

Although there is plenty of evidence that many different chemical treatments

can kill mites, there is disappointingly little evidence that they unreservedly improve the survival or productivity of infested colonies. Recent tests (Cox *et al.*, 1988, 1989b; Wilson *et al.*, 1990) showed that severely infested colonies taken to Nebraska and treated with menthol in spring survived the following winter better than similar untreated colonies; but they produced less honey and grew less than untreated colonies even though their infestation declined sooner and to somewhat lower levels than in the untreated ones. In Britain, autumn treatment with materials that effectively killed mites led to much earlier deaths than usual in winter of infested individuals (Bailey and Carlisle, 1956). This can kill moderately infested colonies that otherwise could survive the winter and lose much of their infestation during a following good season.

The popular attitude often seems to be that any disadvantages of treatments cannot be worse than infestation, so eradication of infestation becomes the only desired objective. The economy of this must be questioned. Some suppression of infestation in spring and early summer, well short of likely damage to bees, seems the most advisable treatment with acaricides; and no treatment at all may well be more profitable when there are only average levels of infestation, especially where bees usually experience good nectar-flows. More rigorous treatment may be advantageous only if bees become severely infested (see Section 4.).

3. Resistant strains of bees

There is no evidence that strains of bees more resistant than average have emerged spontaneously or have ever been selected and maintained by beekeepers. Strains could be selected by the laboratory method of Gary and Page (1987; Chapter 7, I.E.2(a).) more conveniently and in many ways more efficiently than by field trials with colonies; but bees selected by such means would not necessarily have the best foraging characteristics, or other possible qualities that suppress the spread of infestation within colonies.

4. The effect of beekeeping methods on infestation

Infestation is aggravated by procedures that check the growth and foraging of colonies. Infested colonies that suffer periods of queenlessness in early summer become more severely infested than colonies with queens (Bailey and Lee, 1959). The numbers of the external mite *Acarapis vagans* also increase in queenless colonies (Schneider, 1941). The numbers of the other *Acarapis* species probably increase similarly (Chapter 7, II.).

Keeping too many colonies for the available forage, and the frequent closure of hives may be expected to increase infestation by increasing contact between the older, more infested bees, which would normally be out foraging, and young susceptible individuals. Such events are common in modern beekeeping,

particularly in the package bee, queen-rearing, migratory beekeeping and pollination industries, which are often very intensive, some especially so in southern USA. These activities can lead to high colony densities (see Lovell, 1963) for protracted periods when nectar-flows are inadequate, or competition between colonies makes them so, and when young bees are being reared.

Much infestation could be taken to regions with cold winters in package bees from warmer areas or in colonies sometimes overwintered there by migratory beekeepers (Chapter 7, I.E.2(c).). Such colonies would be expected to overwinter less well than indigenous ones.

Beekeepers with many colonies could increase their resistance to infestation by dispersing them more widely and in smaller groups, or apiaries, than they usually do. The aim should be to avoid or diminish local overstocking (see Dadant, 1963).

B. Varroa jacobsoni

1. Manipulative treatment

Ruttner and Ritter (1980) advocate a method of decreasing the amount of brood in infested colonies to prevent migrating females of *V. jacobsoni* from finding protection in sealed cells against chemical treatments. The method is to confine the queen on one or two empty brood-combs, preferably of drone cells, surrounded by combs full of sealed stores or by frames of wax foundation on which the queen does not lay. The queen can be prevented from returning to the main mass of brood by means of wire grids ("queen-excluders") with slots wide enough for workers but not queens to pass through. The combs on which she lays are removed, when the brood in them has been capped, and are replaced with empty ones. The process is continued for about a month by the end of which the colony has no brood except in the combs on which the queen is confined. These trap most of the migrating mites that have escaped chemical treatment and they are removed and destroyed with the combs. The method is ingenious, but it destroys a month's production of brood. This, the labour involved, and the aggravating effect the method would have on other infections, such as those by *Nosema apis* and *Acarapis woodi*, are severe disadvantages.

2. Chemotherapy

Even more chemicals have been tested against *V. jacobsoni* than against *Acarapis woodi* (International Bee Research Association, 1986). A similarly wide spectrum of unrelated chemicals, ranging from the simplest to the very complex, will selectively kill mites in bee colonies. The basic reason for this may be the same as that suggested for their activity against *A. woodi*

(Section A): individuals of *V. jacobsoni* are much larger than those of *A. woodi*, but their surface area relative to their volume is still about ten times greater than that of bees.

Several acaricides have been officially approved in Europe (Ritter, 1988c), but even the best of these are not harmless for bees (Henderson, 1988). Not unexpectedly, acaricides that are active against *A. woodi* also attack *V. jacobsoni*, but a serious difficulty with the latter is the failure of vapours to reach mites within capped brood cells. This can be circumvented, perhaps most effectively when old queens are being replaced (Lupo and Gerling, 1987). The old queen is caged for 10 days in her colony and then replaced with a queen-cell: the new queen begins to lay eggs after about a further 10 days and the colony, now without capped brood, is fumigated on two successive days with an acaricide. Almost total eradication of mites has been achieved this way.

Systemic acaricides, given in food to bees, that could reach mites via the larval food in capped cells would be ideal. Those developed so far do not reach capped brood effectively and are fairly toxic for bees, although they are among the best acaricides (Koeniger and Fuchs, 1988; Neuhauser and Krieger, 1988; Ritter and Schneider-Ritter, 1988).

Formic acid vapour, applied as described in Section A, on four occasions at four day intervals, killed most mites in tests in Germany, including those in capped cells (Hoppe *et al.*, 1989).

Laboratory tests showed that migrating mites are repelled by geraniol (Hoppe and Ritter, 1988), which is the main component of the Nasanov gland secretion of foraging bees. This may explain the preference of mites for young nurse bees, which keeps them near the brood and from being taken out into the field on foragers. Disorientation of mites in colonies by the application of geraniol, which would seem harmless to bees, is a possible way of slowing their spread. Suitably arranged sources of geraniol and of the attractants identified by Le Conte *et al.* (1988) in extracts of drone larvae might form the basis of efficient traps for mites in bee hives.

Many believe that catastrophe throughout continental Europe has been averted only by prompt and effective chemical treatments. The absence of any noticeable fall in honey yields and number of colonies in West Germany since the arrival of the mite (Fig. 43; Ritter, 1988b) might be attributed to this. However, if such measures are so effective, and *V. jacobsoni* is otherwise so destructive, controlled experiments showing a greatly improved survival and productivity of treated colonies would be reassuring. Otherwise, the continuing widespread infestation could leave the impression that the mite is less damaging than has been supposed, and that coincident, undiagnosed and independent disorders are often being attributed to it; or that secondary pathogens, which are spread or activated by the mite but are not always present (Chapter 3; Chapter 4, IV.), cause most harm. Any of these possibilities

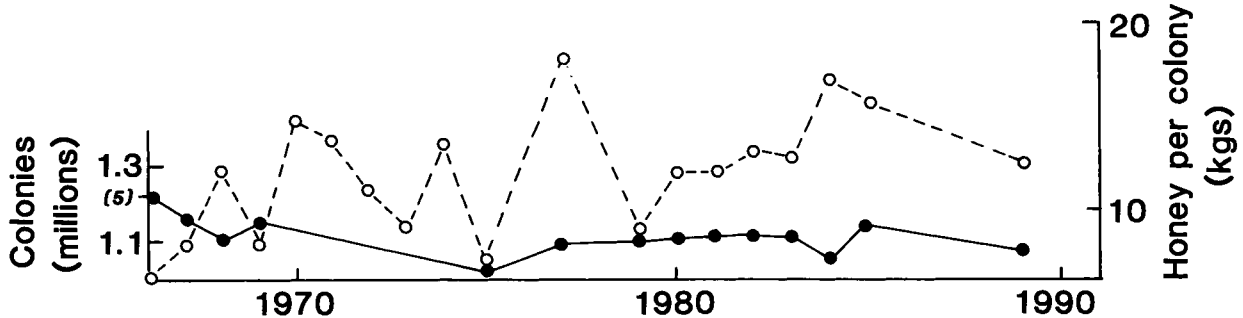

Figure 43 The numbers of colonies (●) and the honey yield per colony (○) in West Germany. (5); 5.0 colonies/km^2. (From Anon., 1982, 1989.)

would greatly increase the difficulty of demonstrating unequivocally the damage done by infestation. These matters must be resolved if there are to be no reasonable doubts about the value of measures taken against infestation.

C. Tropilaelaps clareae

1. Manipulative treatment

Woyke (1985a) describes three simple methods that depend on the fact that *T. clareae* is wholly dependent on the presence of brood:

1. The queen is caged for 21 or more days until all the brood has emerged.
2. The queen is caged for about 9 days until all the brood is sealed. This is then uncapped and shaken out.
3. All brood is taken out and destroyed.

All mites disappear 3 or 4 days after the end of any of these treatments. However, the losses involved would seem unacceptable where early re-infestation is likely.

2. Chemotherapy

Acaricides that selectively kill *V. jacobsoni* and *A. woodi* are also effective against *T. clareae* (Woyke, 1987), but most individuals of *T. clareae* are protected within capped brood-cells. Interestingly, systemic acaricides fed to bees work well against individuals of *T. clareae* that are on adult bees, which suggests that the mites take food from the mouths of the bees, similarly to *Braula coeca* (Ritter and Schneider-Ritter, 1988).

A single treatment of formic acid vapour, applied as described in Section A, has been found very effective and, unexpectedly, it kills at least some of the mites in capped cells (Hoppe *et al.*, 1989).

VI. INSECT PESTS

A. *Wax Moths*

One of the most efficient fumigants against wax moths is ethylene dibromide (Krebs, 1957). Boxes of comb should be enclosed in plastic bags into which the liquid ethylene dibromide is added. Between 20 and 100 g of ethylene dibromide per 1000 litres of volume to be fumigated has been recommended.

However, it is poisonous, vesicant and a suspected carcinogen (David, 1963). Methyl bromide is very effective too (Roberts and Smaellie, 1958), but is dangerously poisonous to man and is a gas at normal temperatures, which makes it less convenient than ethylene dibromide.

Paradichlorbenzene is safe for man and its crystals give off vapour which quickly kills both adults and larvae of wax moths at ordinary temperatures. Eggs are much more resistant to it, however, and combs are repellent to bees for a short time after treatment.

Acetic acid, as used for fumigating combs against contamination by *Nosema apis* or *Malpighamoeba mellificae* (Section IV.A.1.), quickly kills eggs and adults of wax moths. However, larvae, especially the largest of *Galleria mellonella*, are resistant, particularly when they are buried in comb debris.

Formic acid, recommended for treating mite infestations (Section V.) kills young wax-moth larvae (Hoppe *et al.*, 1989).

Heating combs at 49°C for 24 h at 50% relative humidity, as suggested for disinfecting them of *Nosema apis* spores (Section IV.A.1.), kills all stages of wax moths (Cantwell and Smith, 1970).

Cooling combs to temperatures between 0°C and −17°C, for a few hours to several days, according to temperature and bulk of material, kills all stages of wax moths without harming combs or stored honey (Burges, 1978).

Preparations of *Bacillus thuringiensis* are effective and specific against wax-moth larvae, and proprietary formulations are available for beekeepers to apply.

B. *Other Insects*

According to Boiko (1948) adult flies that cause apimyiasis rest on hive roofs, especially when these are light in colour, whereas bees do not, and a recommended control measure is to paint the roofs with a persistent insecticide.

Naphthalene sprinkled on paper on the hive floor is said to kill oil beetle larvae without harming bees (Minkov and Moiseev, 1953), and a long established practice said to relieve queen bees of infestation by *Braula coeca* is to expose them briefly to tobacco smoke.

VII. INSECTICIDES

The only satisfactory way to avoid losses caused by insecticides, apart from using only selective ones that are harmless for bees, is to keep bee colonies 2 miles or more away from areas that are to be sprayed, and to spray crops when bees are not attracted to them. These are matters requiring close and

friendly relationships between beekeepers and farmers rather than legislation. When colonies cannot be moved from an area to be sprayed they can be protected by covering them, including the hive entrance, during daylight hours, with coarse sacking material. The sacking should be kept soaked with water to keep the colonies as cool as possible (Wilson *et al.*, 1980).

11
CONCLUSIONS

A wide variety of specific pathogens are enzootic in honey bees, and most of them are perpetuated as inapparent infections. Colonies and even individual bees that are infected with certain, sometimes several pathogens frequently seem outwardly normal for an indefinite period. Accordingly, it is often difficult to identify the cause of losses and disorders; and this has led to much confusion and many false diagnoses, which have been compounded by a common tendency to make scapegoats of newly recognized but widespread parasites, even when their incidence within colonies is low. Particularly misleading in this matter are the commonly used terms "Nosema disease", "Amoeba disease" and "Acarine disease". They indicate the first three common parasites that were identified in adult bees—*Nosema apis*, *Malpighamoeba mellificae* and *Acarapis woodi*—and, to many beekeepers, they mean a sickness with outward signs. The parasites are indeed harmful, as has been discussed in earlier chapters, but they have never been shown to cause any outward sign of disease, although they often coincide with disorders, some still of unknown cause, that do cause such signs. The damage done by the widespread inapparent infections of bees—essentially a shortening of the lives of adult individuals—is probably considerable; but it is usually obscured by the many inevitable and continuous losses of healthy but spent adult bees and their constant replacement, especially during the active season.

Understandably, signs of severe disease were the ones that were first investigated; and when the microbial aspects of honey bee pathology began in the late nineteenth and early twentieth century, they gave rise to the belief that pathogens of bees always cause diseases as severe as those that had been recently diagnosed in silkworms and other domesticated animals. In fact, apparently healthy colonies of bees resemble all other populations of wild animals, including pest insects, by sustaining many different infections and in most circumstances by not being crippled by them. However, the balance achieved by even the best adapted strains of bees with any of their pathogens is an uneasy one and can be quickly upset by certain factors, either environmental or to do with beekeeping, that lead to intolerable losses. Many

writers lump these predisposing factors under the term "stress", which here means no more than "unfavourable circumstances" and explains nothing. The factors must be resolved in some detail if a satisfactory explanation is to be made of why any one type of enzootic pathogen sometimes multiplies and spreads more than usual, and if improved control measures are to be devised. Many, perhaps most details are still unknown, but some principles are discernible.

There is no evidence that insects are protected from infections by acquired immunity. The spread of infections between bees is limited by an innate immunity of individuals during much of their lives, by their short life-span and replacement with healthy individuals, and by events that decrease the chance of contact between pathogens and susceptible healthy individuals. Pathogens that spread contagiously between live bees are especially hindered when the normal activities of colonies, particularly foraging, are intense. The older adult bees, which are those most likely to be infective and are the foragers, then have least contact with their younger, healthier and most susceptible hive mates. Less directly than this, foraging activity also helps to clear away the resting stages of other kinds of pathogens from the combs by increasing the activity of cell-cleaning bees which prepare the combs for food storage and brood-rearing.

Foraging is often curtailed by bad weather or crop failures, which are not controllable. However, beekeepers frequently inhibit the foraging of their bees to some degree, especially by overstocking, sometimes of necessity. Colonies then do not flourish as well as they might and any common enzootic pathogens that become more numerous are often blamed as the primary cause of their poorer performance. Because of this, many beekeepers have come to believe that food shortage lowers the resistance of bees to infections and, therefore, that feeding sugar to their colonies will be curative. On the contrary, it can aggravate contagiously transmitted pathogens, and there is no evidence that it has any beneficial effect other than saving colonies from starvation. The surviving bees flourish in subsequent nectar-flows, and it is this increase of normal activity that mainly helps them to suppress infections.

Many beekeepers also believe that drifting and robbing by adult bees, which occur particularly between crowded colonies, increase the degree to which these become infected by enzootic pathogens. The idea has probably been strengthened by the facts that pathogens, drifting and robbing all tend to increase with increasing colony densities. However, the transmission of any type of pathogen between colonies that are already infected is of little or no consequence. The important factors then are those that control its multiplication and spread within colonies.

Colonies in natural habitats are probably more widely dispersed on the whole than when kept by man, and so they are less likely to suffer from a

shortage of nectar and pollen, and the consequent inactivity that in turn leads to the spread of pathogens within them. Furthermore, nests of wild colonies that become weak and die are less likely than beekeepers' colonies to be found and ransacked or occupied by other bees before they are found and destroyed by scavengers. Such events again decrease the chance of contact between healthy bees and pathogens. It is unnecessary, often impossible, for them to be separated absolutely, but when the balance between the spread of a pathogen and the forces that oppose it is tipped sufficiently in favour of the host, the natural system will itself do the rest and disease will be controlled, or even completely suppressed. Pasteur was able to eradicate pebrine from the silkworm because he could select healthy stocks of insects and then keep them easily and permanently in isolation. This can rarely be done, and then often only with difficulty, even with domesticated animals. It cannot be done with feral animals such as bees, as has been explained previously (Chapter 2, II.); so it is unreasonable to expect to eradicate their diseases easily. Nevertheless a great deal can be done to avoid aggravating them and to assist the natural mechanisms that oppose their spread.

Beekeepers began seriously to interfere with the natural processes that decrease the numbers of resting stages of pathogens on combs when they changed their hives with fixed combs, which had to be destroyed when their honey was extracted, for those with moveable combs, which are durable. These combs are frequently stored away from bees, to be returned to them when they need more space. This interrupts the natural cleaning process and reinoculates pathogens into the colonies when the combs are returned. Often this occurs too late in the season for the bees to recover from the infection as efficiently as usual. The efficient sterilization of combs or even the destruction of those known to be severely contaminated is the obvious solution.

The popular preference, or hope, has always been to treat disease with drugs; and the advent of effective specific ones, particularly the sulphonamides and antibiotics that act so dramatically against bacteria, seems to have justified this desire for spectacular, almost magical results. Various nostrums have always been recommended, long before the advent of effective antibiotics, even by Virgil (70–19 B.C.) who advised that various herbs and wine should be fed in honey to ailing colonies; but there is no evidence that any are of benefit. Like so many alleged remedies, their apparent effectiveness most probably depends upon the innate power of animals to recover from disease, sometimes in spite of treatment. Indeed, the damage to bees caused by many treatments has often been believed to be the result of infections too advanced to be cured and has increased the notoriety of bee diseases. This applied particularly to *Acarapis woodi* during the early part of the century and probably had much to do with the alarm about *Varroa jacobsoni* more recently.

Even effective antibiotics can be toxic to bees and are often seriously

misused. Furthermore, it can be pointless, or at least inadvisable, to use them against diseases that usually decline spontaneously when they are known to have reached their seasonal peak. Antibiotics are then least likely to affect the course of infection or to prevent contamination of comb by dormant organisms, and they are most likely to contaminate honey harvested for human consumption. Their use against severe, or potentially severe, diseases such as American or European foulbrood is inadvisable when the incidence of disease is very low. It is then more economical to destroy the few colonies that become diseased. Otherwise, the use of antibiotics, which do not eradicate infection, allows pathogens to spread undetected and leads to dependence on frequent and widespread treatment.

Virus diseases seem to pose a less tractable problem than the rest. Viruses of bees do not appear to have resting stages. They do not survive long outside the living tissue in which they multiply, and, in common with all viruses, they are not susceptible to treatment with available antibiotics. Knowledge of their ecology is scanty, but there are indications that some spread faster between individual bees when foragers are confined to their colonies, or are partially restricted at least, at times when they would usually be flying freely. Some virus diseases are secondary infections, dependent upon primary agents such as *Nosema apis* or *Varroa jacobsoni*, and then make the control of these parasites more important than when the viruses are absent. Although no direct therapies are yet known for virus infections, an awareness of these is needed if only to avoid mistaken or inadequate diagnoses and to avoid the importation of exotic types and strains. Further knowledge of them is needed, and their successful management could well be the reward of a better understanding of their ecology.

All diseases are influenced by genetic factors, but there is no evidence that any strains of honey bees have genes that give them immunity from any of their known pathogens. Beekeepers have occasionally claimed that their bees were immune because the bees did not succumb, or because they recovered quickly after being exposed to certain diseases. The beekeepers were impressed, because they have been led to believe that bees are usually very susceptible, whereas their observations confirmed that bees have considerable powers of resistance and recovery. Even were immune bees to exist, it would be very difficult to replace common strains with them before virulent mutants of the pathogens found their way back from the reservoir of susceptible bees. The genetic variability of pathogens and the possibility of the inadvertent selection of more virulent ones is frequently overlooked. Combs contaminated with pathogens, possibly of greater virulence than usual, are frequently removed from dead colonies and used again instead of being destroyed, as many would be in nature by scavengers. Colonies that are ailing when others are not, and that are often helped to survive by beekeepers, may also be

infected with unusually virulent pathogens which would be better destroyed.

There is no doubt that bees can be selected with resistance that is greater than average towards disease, as has been demonstrated by Rothenbuhler and his colleagues with American foulbrood. It seems significant, however, that resistance towards this disease, which is the most likely of all diseases to kill a bee colony, is at least partly determined by recessive factors (Chapter 10, II.A.5.). This suggests that death of infected colonies has enabled the species to survive better than has natural selection for resistance. Whatever the reason, it makes the maintenance of resistant strains more difficult than if resistance were due to dominant genes. Attempts to find strains of bees more resistant to disease will inevitably continue, but the cost of maintaining and propagating them has to be weighed against the cost of the disease. Susceptibility to a disease that is slow to spread between colonies, is of low incidence and is easy to see could well be preferable to resistance that allowed it to become widely distributed in a form often difficult to detect.

It is a common belief that pathogens eventually become avirulent, or nearly so, after long association with a host, and some honey bee pathogens have been thought to provide examples. However, a pathogen will evolve in the way that best secures its chances of survival. Virulence may be essential for it to achieve this. For example, *Bacillus larvae* must kill the individual it infects in order to form durable and infective spores. In nature, before beekeeping began to influence its distribution, its spread between colonies must have been very slow, otherwise all bees would have been destroyed. The bacillus may originally have been localized to cool habitats, where wax moths do not destroy combs as quickly as they do in warm climates. At the other end of the scale, *Acarapis woodi* is only slightly harmful to adult bees. Its life cycle is long, relative to that of its host which has to survive in order to transmit migrating mites to other bees. Greater virulence would be disadvantageous for the mite, although it remains sufficiently harmful to damage or even kill the relatively few colonies that become severely infested.

The artificialities of beekeeping will no doubt increase as agriculture develops, particularly as it grows in scale and gives extensive areas of single, nectar-yielding crops for limited periods. This encourages beekeepers to move their bees from one crop to another, so inhibiting the development and normal activities of colonies, and to keep them too crowded in apiaries, especially between the periods of major nectar-flows. Such practices encourage the spread of infections within and between bee colonies. Furthermore, the common wish of beekeepers to import bees that are alleged to be superior to their own is easily gratified with the aid of modern transport, but little heed has been paid to the dangers of introducing exotic diseases or unusual strains of pathogens, especially of viruses, which are not easily diagnosed. For these reasons more attention needs to be paid than in the past to the prevention and suppression of diseases. The difficulties to be overcome may be great, but so is the room for improvement.

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