

The role of *Varroa* and viral pathogens in the collapse of honeybee colonies: a modelling approach

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Summary

1. The ecto-parasitic mite *Varroa destructor* is a serious world-wide pest of the honeybee *Apis mellifera* and has been linked with the death of millions of colonies, although its role in colony death has remained elusive.

2. A simulation model was developed to explain the link between the mite and collapse of the host bee colony, given that colony death does not always occur. We investigated the effects of two pathogens, deformed wing virus (DWV) and acute paralysis virus (APV), vectored by the mite, on the host colony.

3. Two previously published simulation models, a bee and a mite, were combined and adapted for use in temperate climates with a variety of bee diseases. The model was constructed using Modelmaker® software, which allows the progression of a disease in the host colony to be followed daily.

4. The population dynamics generated by the model were similar to those observed in a natural honeybee colony. When DWV- or APV-transmitting mites were introduced into the colony, its adult worker bee population collapsed either during winter or spring for DWV, or autumn to spring for APV. This corresponds well with field observations of colony death in Europe.

5. The model revealed that DWV initially had little effect on the colony but during late summer, as the population of DWV-transmitting mites increased, the virus caused a reduction in the number of healthy young bees entering the overwintering population. This imbalance in the age structure of the overwintering bees resulted in the eventual death of the colony during the winter or spring. As few as 2000–3600 mites in autumn could kill a colony.

6. In contrast, APV transmitted by *Varroa* was only able to kill the honeybee colony if a large (10 000+) mite population was already present when an overt APV infection occurred. It was difficult for APV to become established within the bee population due to it causing rapid host death.

7. The model predicts that the less virulent DWV will become more widely established than the highly virulent APV, and that mite control measures need to be taken prior to the production of overwintering bees.

Key-words: *Apis mellifera*, bee viruses, colony collapse, disease, *Varroa destructor*, *Varroa jacobsoni*.

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Introduction

Social insect colonies are often composed of tens of thousands of individuals, of many overlapping gener-

ations, and so have evolved feedback mechanisms at many levels to allow the colony to function as a cohesive unit. In honeybee colonies, mechanisms such as variation in egg-laying rates (Allen 1960) and brood cannibalism (Fukuda & Sakagami 1968) help regulate colony population growth. This adaptive demography with respect to seasonality and resource management allows a colony to persist in a variable environment (Seeley 1995).

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One aspect of this variable environment is disease. Mortality caused by disease can influence colony demography, by increasing death rate and/or reducing birth rate. In extreme cases the colony can be eradicated. The effects of diseases on insect colonies are complex, acting both at the level of the individual and the colony. The colony is only threatened if premature death or behavioural change affects many individuals. The mechanisms by which a colony either succumbs to or overcomes a disease remain largely unstudied, with limited understanding of the population dynamics of large social insect colonies and little detailed information on the epidemiology of their diseases.

One way to further our understanding of the colony response to disease is simulation modelling of the population dynamics of an insect colony (Archer 1981; Martin 1991) in which a disease is also being transmitted. This study represents the first comprehensive attempt to model the progression and effects of any disease in a social insect colony. The system involves two viral pathogens, deformed wing virus (DWV) and acute paralysis virus (APV), which are both transmitted by an ecto-parasitic mite, *Varroa destructor* Anderson, while feeding on its host, the honeybee *Apis mellifera* L. [Anderson & Trueman (2000) demonstrated that *Varroa jacobsoni* is a complex of at least two species: *V. jacobsoni* and *V. destructor*, of which only the latter is a pest of *Apis mellifera*.]

BIOLOGICAL BACKGROUND

Host, the honeybee Apis mellifera

Honeybees are both ecologically and economically important. Due to their long association with humans they are the best-studied social insect (Winston 1987). In temperate climates during times when nectar and pollen are abundant (spring and summer), a colony consists of a single reproductive queen, between 20 000 and 60 000 adult workers, and 10 000 and 30 000 individuals at the brood stage (eggs, larvae and pupae). The brood stages are reared individually in hexagonal wax cells. The cell is open during the egg and larval stage and sealed during the late larval and pupal stages. The cell remains sealed until the fully formed adult chews a hole in the wax capping of the cell, so releasing itself. The queen and 8000–15 000 adult workers overwinter, feeding solely on honey stored during the summer.

As each colony is a dense group of individuals, honeybees would seem particularly vulnerable to pests and pathogens. Although honeybees have many defences against diseases, such as hygienic behaviour or the production of anti-microbial substances (Seeley 1985), colonies still suffer from a number of diseases and pests (Bailey & Ball 1991; Schmid-Hempel 1998).

Vector, the mite Varroa destructor Anderson

Until the 1950s *Varroa jacobsoni* Oud., an ecto-parasitic

mesostigmata mite, was found only in association with its natural host, the Asian hive bee *Apis cerana* F. This bee has various behavioural and physiological adaptations that limit *Varroa* population growth (Rath 1999). However, around 1957 *V. jacobsoni*, which is now called *V. destructor*, shifted host to the western honeybee *A. mellifera*, and since then has spread rapidly throughout most of the world (Oldroyd 1999). *Varroa* is now the most important pest of *A. mellifera* and has been linked with the world-wide collapse of millions of colonies (Martin 1998). An adult female *V. destructor* lives either attached to an adult honeybee (phoretic phase) or reproducing within a sealed drone or worker brood cell. To reproduce, a female mite enters a brood cell just prior to sealing and lays three to five eggs, of which only the first is male and the rest female (Martin 1994). The eggs develop into adults within the sealed brood cell, where local mating occurs, typically between brother and sisters. The adult female mites and any offspring are released into the colony when the bee emerges from the cell. The male and any immature females die once the bee has emerged. All stages of the mite feed on bee haemolymph, which is obtained by piercing the bee cuticle with specialized mouthparts. In *A. mellifera* colonies in temperate climates, *V. destructor* populations increase unhindered until the host colony collapses. However, although colony collapse is associated with *V. destructor*, no direct link between the actual mite population and colony collapse has been found (Martin *et al.* 1998). Although eight collapsing colonies had estimated populations of between 2600 and 16 000 mites, seven surviving colonies had a similar sized mite populations (2500–15 000). This strongly suggests that other factors are also involved in the collapse of mite-infested colonies.

Pathogens, deformed wing virus (DWV) and acute paralysis virus (APV)

Fourteen small (17–60 nm) ribonucleic acid (RNA) viruses are currently known in the honeybee (Bailey & Ball 1991). Normally, these viruses persist benignly and rarely appear to cause colony mortality. In *Varroa*-free colonies, detectable levels of APV have only been found in adult workers from Belize in 1976 (Bailey, Ball & Perry 1981) and 1982 (B. Ball, personal communication), while DWV has been detected in adult workers in several *Varroa*-free colonies in the UK and South Africa in 1997 and 1993, respectively (B. Ball, personal communication). However, in colonies severely infested with *V. destructor*, APV has been found in dead adult workers from Germany (Ball 1985; Ball & Allen 1988), Russia (Batuev 1979) and the USA (Hung *et al.* 1996). DWV now appears to be the most widespread virus, occurring in infested colonies in the UK (Martin *et al.* 1998) and elsewhere in Europe (Kulincevic, Ball & Mladjan 1990; Topolska, Ball & Allen 1995; Nordstrom *et al.* 1999). Both APV (Batuev 1979; Ball 1989) and DWV (Bowen-Walker, Martin & Gunn

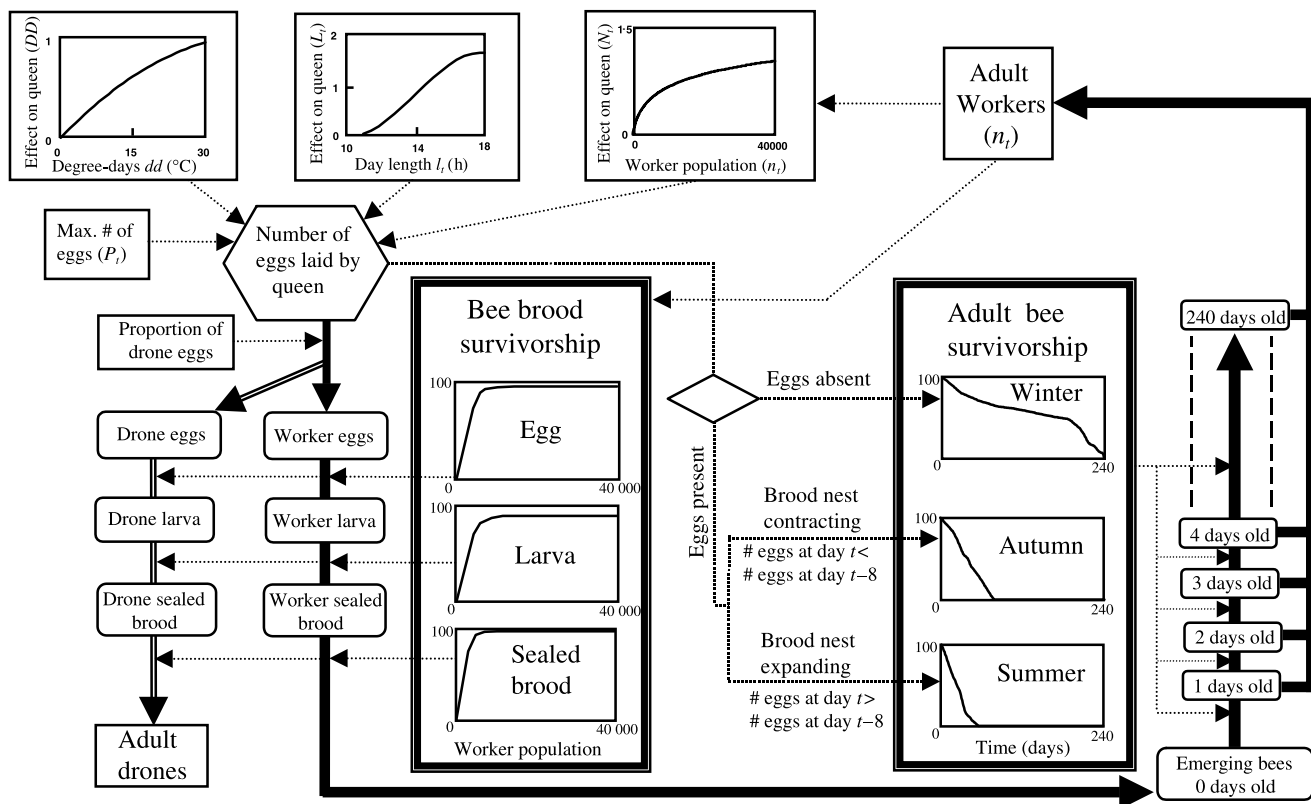


Fig. 1. Basic bee model flow diagram. Solid lines indicate the flow of individuals between compartments and dotted lines indicate the influences.

1999) can be transmitted between bees by the feeding activities of *V. destructor*. When these viruses are artificially injected into bee pupae, they can cause wing deformity (DWV) or death (APV) (Bailey & Ball 1991). Adult bees injected with 10^2 particles of APV die within 4–8 days (Bailey & Gibbs 1964; Bailey, Ball & Perry 1981). Detailed studies on the effect of DWV on the survivorship of individual honeybees have recently been completed (S. Martin, B. Ball & N. Carreck, unpublished data). Results showed that when DWV is transmitted to pupae by feeding, pupal mortality increases and the longevity of the adult workers is reduced. However, the longevity of adult workers infected after the pupal stage is unaffected. All infected bees become reservoirs of viable virus until they die. Both viruses can be spread between colonies by the movement of infected adult bees (e.g. drifting) and their mites or by beekeeping practices that move sealed brood or adult bees.

Models

The models have been developed by dividing the honeybee or mite life cycle into compartments, each representing a measurable quantity, such as the number of eggs laid or mortality rate. These are then linked together in a biologically realistic manner using Modelmaker® software (Cherwell Scientific Publishing, Oxford, UK; www.modelkinetix.com) so that individuals flow through the population on a daily basis.

For example, DWV-infected bee pupae develop into infected adult bees that have a reduced survivorship. This modular approach allows the demographic structure of the honeybee colony to be interrogated on any day, and allows the direct, accurate, integration of non-linear functions such as survivorship curves. The adapted bee and mite models were each validated by comparing results with those from previous studies. Only then were they linked together so that the effect of DWV and APV on a *Varroa*-infested bee colony could be studied. The model equations are given in the Appendix.

BEE MODEL

The basic bee model (Fig. 1) was adapted from Beepop (DeGrandi-Hoffman *et al.* 1989), a model developed to simulate the population of a honeybee colony, for use in a temperate climate. Beepop uses meteorological data to determine queen egg-laying rates and worker longevity. However, in temperate climates this results in no bee death over the long winter period, which is unrealistic. Also, it is difficult to model the effect of a disease on adult workers using this approach. Therefore, in the current study worker longevity was determined directly from the survivorship curves.

The daily numbers of worker and drone eggs laid by the queen were determined using the equations in Beepop (DeGrandi-Hoffman *et al.* 1989; see the Appendix) in conjunction with meteorological data for

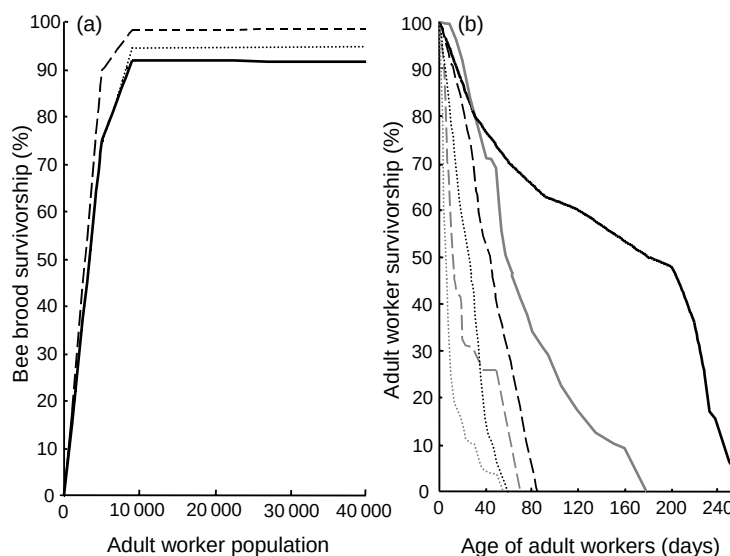


Fig. 2. (a) Survivorship curves for honeybee brood egg (dotted line), larvae (solid line) and sealed brood (dashed line). (b) Survivorship curves for non-infected (black lines) and DWV-infected adult workers (grey lines), depending on the time of year; summer (dotted line), autumn (dashed line), winter (solid line).

1999 from the Institute of Arable Crops Research, Rothamsted Experimental Station, Rothamsted, UK (Fig. 1). To reduce the amount of variability, the mean air temperature for one year was repeated in subsequent years. The size of the colony could be adjusted by altering the maximum number of eggs laid per day by the queen, which was set at 1500 unless otherwise stated. The queen is assumed not to age during the period of the simulation, so there is no decline in her egg-laying rate.

Eggs laid each day develop into adults by flowing through compartments that represent developmental stages (e.g. egg, sealed brood). The length of time in each compartment is determined by the development period of that stage, and the flow rate between compartments by the survivorship probability at that stage. For survivorship curves the values between known data points were interpolated linearly using Model-maker® to create a daily look-up series. This allows non-linear data to be entered directly into the model without an explicit equation. Because the size of the adult population has an important role in brood survival that is not related to the season of the year (Garofalo 1977), data from Fukuda & Sakagami (1968) and Harbo (1986) were used to construct brood survivorship curves for each developmental stage (Fig. 2a). For colonies of more than 9000 adult bees (Harbo 1986), the brood survival rate was 94%, 91.7% and 98.5% for the egg, larvae and sealed brood stages, respectively (Fukuda & Sakagami 1968). There are few observations of levels of brood survival in small colonies. Colonies with a population of 4500 adult bees were able to rear more adult bees (Harbo 1986), while this was not possible in a colony of 3000 bees (Fukuda & Sakagami 1968). Therefore, survival values of 75% for the egg and larvae and 90% for the sealed brood were assumed at a

colony size of 5000 bees. These values then decreased linearly to pass through the origin, as brood survival will be zero when no bees are present (see the Appendix for equations). The adult worker population therefore affects both egg laying and brood survivorship. These are critical feedback mechanisms that determine colony population dynamics.

After emergence each cohort of adult bees passes daily between compartments. The proportion that survives to pass to the next compartment is determined by a series of adult worker survivorship curves. For healthy bees in summer, autumn and winter, data derived from Fukuda & Sekiguchi (1966) and Free & Spencer-Booth (1959) were combined (Fig. 2b, black lines). Bees infected with DWV as pupae in summer, autumn and winter had reduced survivorship (Fig. 2b, grey lines), as shown recently by S. Martin, B. Ball & N. Carreck (unpublished data). Because workers in temperate regions are known to exhibit different survivorships depending on the number of individual brood present, worker survivorship was linked to changes in the amount of brood present rather than directly to the time of year. To determine whether the brood was increasing or decreasing, the model compared the number of eggs laid on that day with the number laid 8 days earlier (Fig. 1). The gap of 8 days smoothed out fluctuations in egg-laying rates caused by daily changes in weather from the meteorological data.

No effect of worker population on adult worker mortality is assumed (Harbo 1983). In this study we assume that a colony dies if it falls below a population of 4000 adult workers during the winter, because as the colony size reduces it becomes increasingly difficult for individual bees to maintain an elevated body temperature at low ambient temperatures (Free & Spencer-Booth 1958; Harbo 1983).

THE *V. DESTRUCTOR* MODEL

In previous simulation models (Martin 1998; Calis, Fries & Ryrie 1999), the host honeybee colony was unaffected by changes in the mite population. This was an important limitation (Calis, Fries & Ryrie 1999) and arose because it was unclear how the mites affected their hosts. The earlier mite model (Martin 1998) was adapted so that it could be linked to the bee model and could incorporate relevant viral data parameters. The following changes were made.

1. If an infested bee pupa dies, any mites that invaded that cell do re-enter the phoretic population, as the adult workers normally uncap the cell to remove the dead pupae (hygienic behaviour), so releasing any trapped mites (Boecking & Spivak 1999). However, no progeny are produced from that cell.
2. Each mite leaves its host shortly after it emerges from the brood cell and moves to another uninfested bee (Kuenen & Calderone 1997), where it remains until reproducing or dying. If the host bee dies, mites move to a new healthy host. This is achieved by not linking the mortality rates of the bee to that of the mite. This may be unrealistic during the final stages of colony collapse, but at all other times should produce valid simulations as 75% of mites on a dead or dying bee transfer to a live bee within 24 h (Bowen-Walker & Gunn 1998).
3. The rate that mites invade brood cells was determined using the equation (see the Appendix) derived by Calis, Fries & Ryrie (1999), as this is slightly more biologically correct than the method used by Martin (1998).

VIRUS MODEL

Using published and recent unpublished data, the following assumptions were made.

1. *Varroa* is the sole vector of the pathogen. There is no bee-to-bee or bee-to-brood transmission of DWV or APV. Although this may rarely occur with DWV

(Nordstrom 2000) and certainly occurs with APV (Ball 1985; Ball & Allen 1988), in heavily infected colonies it is the mites that are by far the most effective transmitters of these viruses (Bowen-Walker, Martin & Gunn 1999; Nordstrom 2000).

2. APV and DWV are transmitted to honeybee pupae by virus-carrying mites with 100% and 89% probability, respectively (S. Martin, B. Ball & N. Carreck, unpublished data).
3. Twenty per cent of DWV-infected and 100% of APV-infected sealed brood die in the pupal stage (S. Martin, B. Ball & N. Carreck, unpublished data).
4. Surviving adult workers infected with DWV during the sealed brood stage have reduced survivorship (Fig. 2, grey lines) (S. Martin, B. Ball & N. Carreck, unpublished data).
5. Mites carrying DWV or APV infect all adult workers that they infest. Adults infected with DWV become viral carriers but have unaffected survivorship (S. Martin, B. Ball & N. Carreck, unpublished data). Those infected with APV die within 8 days (Bailey 1965).
6. During mite reproduction, if the invading mite is carrying DWV then the virus is passed on to the mite offspring via the bee pupa (Nordstrom 2000; S. Martin, B. Ball & N. Carreck unpublished data). Although it is unlikely that bee viruses are able to propagate within a mite, the subsequent regular feeding by mites on adult bees will ensure that mites infect, and within a few days receive, viable viral particles from the now infected bee. This effective transmission of DWV from mite to bee, and bee to mite, ensures that a high proportion of the expanding mite population will continue to carry DWV. However, the virulent nature of APV means that the spread of APV throughout the mite population will be very different to that of DWV (see Results and Discussion for details).

Figure 3 shows how the bee and mite models were integrated so that the effects of DWV and APV, or any other mite-associated virus, could be incorporated. To integrate the different survivorship rates caused by the

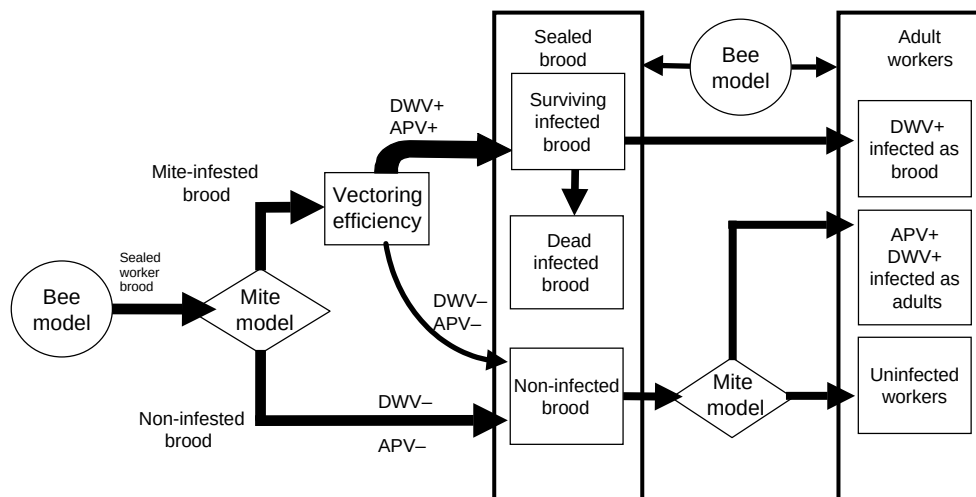


Fig. 3. Integration of the bee and mite models in order to incorporate the epidemiology of DWV and APV.

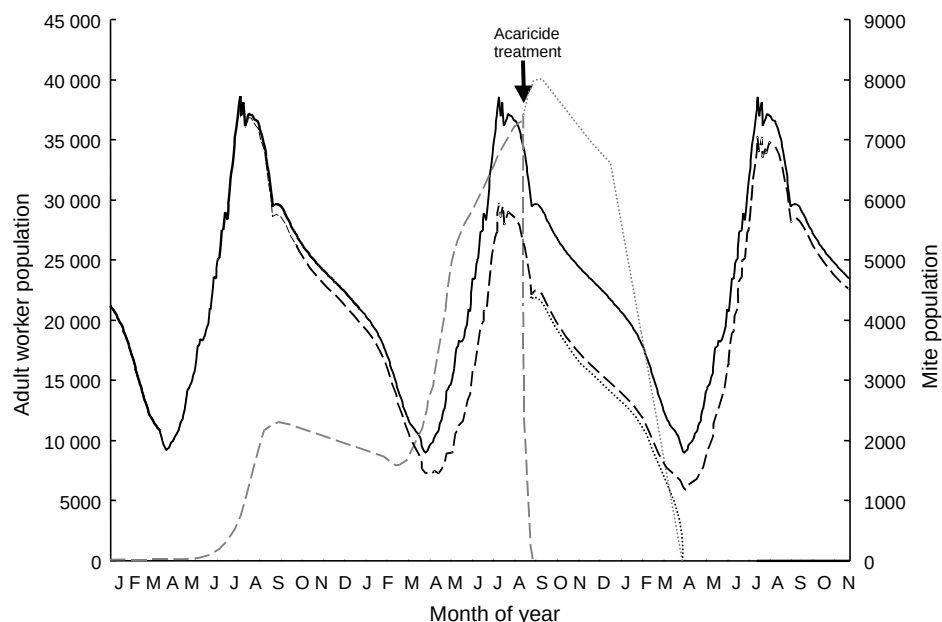


Fig. 4. Adult honeybee worker (black) and mite (grey) populations in three colonies: a healthy (i.e. no mites) colony (solid line) and a colony into which seven DWV-transmitting mites were introduced and allowed to increase naturally (dotted line) or killed with an effective acaricide (dashed line). Note that in the first year the peak mite population of approximately 2000 was just below the survival threshold for a colony of this size, i.e. the colony survived into the next year.

viruses, infected and non-infected sealed brood and adults are divided into separate cohorts that follow parallel paths in the bee model. The proportions of individuals that flow down each path are determined by the level of brood infestation derived from the mite model.

Results and discussion

HONEYBEE COLONY

The bee model was always started by introducing 12 000 bees on 1 January and running it for 3 years with a maximum daily egg-laying rate of 1500. Various numbers of virus-transmitting mites were introduced no earlier than 1 year later. The daily number of eggs laid, time the brood was present (late March to early October, 218 days), subsequent adult bee population (Fig. 4) and the age structure of workers (Fig. 5) in mite-free colonies are similar to actual bee colonies in temperate regions (Bodenheimer 1937; Harbo 1986; Buehlmann 1992). The rate of growth of the mite population ($r = 0.026$) in virus-free colonies was similar to that found in previous studies (0.021 in Calatayud & Verdú 1995; Kraus & Page 1995; Martin 1998; 0.023 in Calis, Fries & Ryrie 1999).

EFFECT OF DWV ON THE HONEYBEE COLONY

When between one and seven DWV-transmitting mites were introduced into the host colony at the start of the simulation, the colony survived for two summers (years) before collapsing during the following winter or spring (Figs 4, 5 and 6). When 15 or more DWV-

transmitting mites were introduced, the colony only survived one summer, again collapsing during the following winter or spring (Fig. 6). This corresponds with field observations showing that, in Europe, colonies die during the winter within 2 years of initial mite infestation (Martin *et al.* 1998; Nordstrom 2000). The time to colony death depended on the initial size of the mite population and the duration of the brood-rearing season. These are the factors that determine how quickly the mite population reached the critical survival threshold.

The build-up of DWV was investigated by comparing the age structure of infected and healthy colonies (Fig. 5). Initially, the proportion of infected adult workers increased slowly because the bee population increased at a greater rate than that of the mite population during the spring and summer. However, as brood rearing diminished at the end of the summer while the mite population continued to increase, there was a sharp rise in the proportion of brood infested with mites (Martin 1998). This resulted in a high proportion of the 'overwintering' bees, which normally survive the long winter period, suffering from a reduced survivorship caused by DWV. This caused a major difference in the age structure of the overwintering bee population, between colonies with and without mites, because adult workers infected with DWV as brood mostly die by mid-winter, leaving insufficient bees to sustain the colony. If the mite population is low, and sufficient bees are present to sustain the colony until the next season, the mites survive the winter on the bees that were uninfected as sealed brood and the DWV is transmitted to these bees. This allows both the mite and DWV to survive the long broodless winter phase.

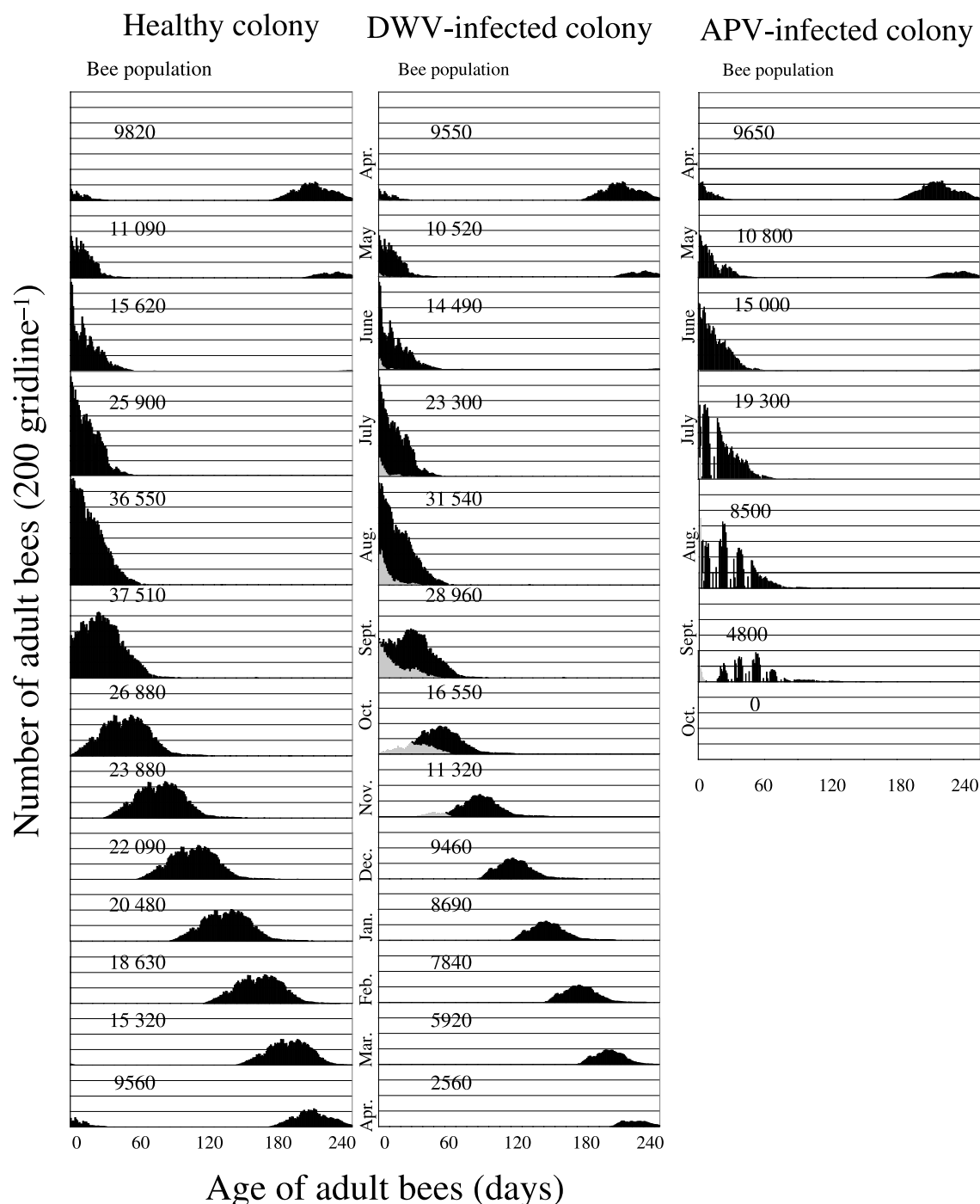


Fig. 5. The age distribution of adult workers from a healthy colony (i.e. no mites), a colony into which five DWV-transmitting mites were introduced 1 year earlier and a colony into which 21 000 APV-transmitting mites were introduced. The adult workers infected with DWV as pupae or with APV as adults have reduced survivorship and are shown in grey.

The chronic nature of DWV explains why it has been so difficult to predict whether a colony will collapse during the winter, because it is not possible for a bee-keeper to see what proportion of the adult workers is infected. A colony can appear healthy and can be full of bees in autumn but still die out during the winter.

successfully. Thus, unlike DWV, mites that vector APV cannot increase in numbers by rearing offspring. Therefore, a large number of mites already need to be present in a colony when an overt APV infection occurs, or is introduced, if sufficient bees are to be killed to cause the colony to collapse. It is possible that these large mite populations assist the rapid spread of APV throughout the mite population. A large mite population will result in many brood cells and adult bees becoming infested with several mites, thus allowing APV to be transmitted from mite-to-mite via the

EFFECT OF APV ON THE HONEYBEE COLONY

The virulent nature of APV causes all infected pupae to die. This prevents the mother mite from reproducing

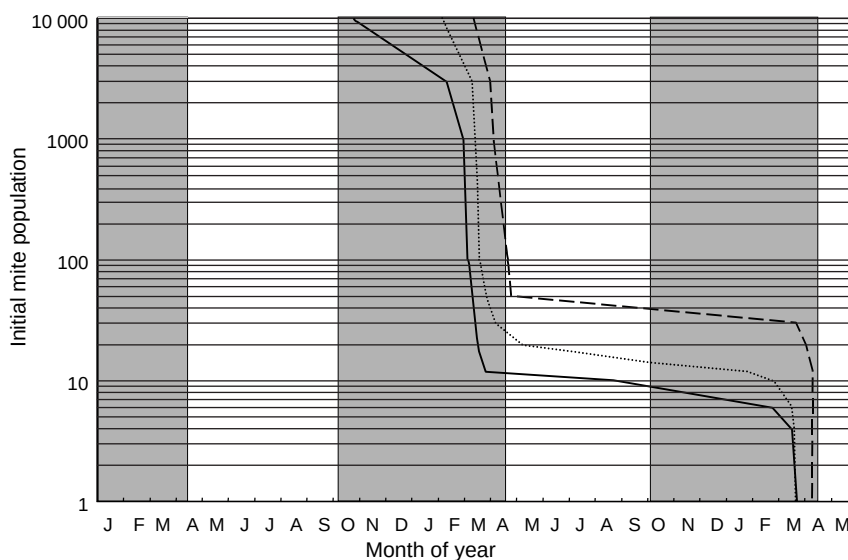


Fig. 6. The predicted time of colony death for three different colony sizes into which 1–10 000 DWV-transmitting mites were introduced. Peak adult worker populations of 30 000 (solid line), 40 000 (dotted line) or 60 000 (dashed line) bees were achieved by setting the maximum daily number of eggs laid at 1300, 1550 and 2000, respectively. The peak sizes of the mite population at which each colony was just able to survive to the next season (survival threshold) was 2000, 3600 and 7000 mites, respectively. The clear and grey regions represents winter/spring and summer/autumn periods, respectively.

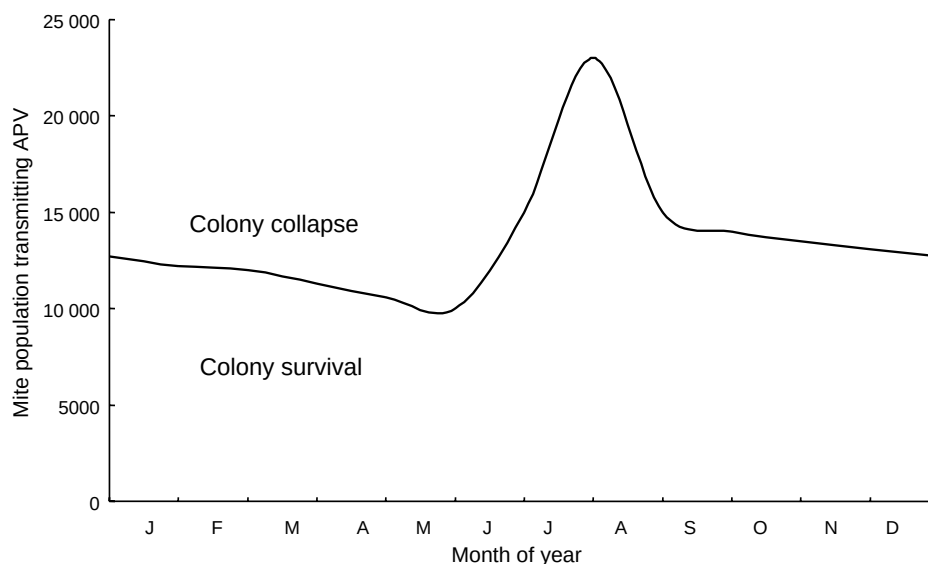


Fig. 7. The size of the mite population transmitting APV during a year that is required to collapse a honeybee colony which has a peak adult worker population of 38 500. Because it is unlikely that APV can spread rapidly throughout the mite population in the absence of bee brood, mites were required to invade a brood cell before the APV transmission cycle could begin.

bee. Direct transmission of APV, i.e. bee-to-bee or bee-to-brood, would further assist the rapid spread of the virus throughout the colony. Figure 7 shows that the predicted size of an APV-transmitting mite population needed to kill a colony varies throughout the year but is always large (10 000+ mites). This finding is supported by a study of colonies killed by *Varroa* in Germany during 1984. Highly infested colonies had estimated mite populations of 20 400 in mid-May (Ritter, Leclercq & Koch 1984) when APV was detected (Ball & Allen 1988), and these colonies died in September of that year. Figure 5 shows the effect of

introducing 21 000 APV-transmitting mites in May into a colony. The acute nature of APV simply removes large numbers of the bee population by killing them quickly. Again the time of death predicted by the model (September) is similar to that observed in the field (Ball & Allen 1988). Unlike DWV, it has clearly been shown that in severely infested colonies larvae in unsealed cells may contain $> 10^9$ particles of APV (Ball 1985; Ball & Allen 1988). This indicates bee-to-brood transmission of APV because *Varroa* does not feed on bee larvae. However, for feeding by nurse bees to cause the death of an adult worker, at least 10^{11} particles of

APV are needed, while only 10^2 particles are needed by injection (Bailey & Gibbs 1964). This may help to explain why diseases transmitted by biting terrestrial arthropods are more severe than those transmitted without vectors (Ewald 1983; Boots & Sasaki 1999). It should be much easier to sustain an outbreak of APV in a *Varroa*-infested colony than in a *Varroa*-free colony, as the mite is a far more effective vector than are nurse bees. It follows that, if a natural outbreak of overt APV occurred in a colony that contained a large mite population, then the mites would greatly increase the effective virulence of the virus by transmitting it directly into the haemolymph of the honeybee pupae and adult workers. If sufficient mites were present this would cause colony death. The rapid movement of mites from dying bees to healthy ones would again enhance virus spread. However, it would still be difficult to sustain the APV outbreak from colony-to-colony because the APV-carrying mites cannot reproduce and APV kills infected adult workers so rapidly that they have little opportunity to drift into another colony, which would also have to be hosting a large mite population. This would only occur in areas where the mite population has been allowed to develop rapidly (i.e. in recently established areas where the lack of effective mite control by beekeepers is common) or the establishment of DWV or other viruses that kill the colony at much lower mite populations has not yet occurred.

Because both of the above factors appear to occur for a fairly short period of time, this may explain the past pattern of occurrence of APV and DWV in *Varroa*-infested colonies. Most reported cases of APV in *Varroa*-infested colonies appear to have occurred soon after the establishment of the mite in an area. Later, the less virulent DWV appears to become widely established and becomes the main virus in *Varroa*-infested colonies.

MITE THRESHOLDS AND TIMING OF TREATMENT

Because it is impossible to eradicate *Varroa* even from a closed population (Sampson & Martin 1999), beekeepers must manage the mite populations within their own colonies. The peak mite population during the previous year at which the colony can just survive into the next year (survival threshold) is given in Fig. 6 for each colony size. This indicates that a relatively small number of DWV-transmitting mites (2000–3600) can cause the collapse of colonies with 30 000–40 000 adult workers. This is because in temperate climates it is the size of the overwintering population that is critical, and in small colonies this may be fewer than 10 000 adult workers. Therefore, if each mite infects only two of the developing workers destined to be part of the overwintering population, at least half of the overwintering population will be dead by December. This explains the threshold values of 2500–3500 that have

been found in previous studies (Delaplane & Hood 1997; Martin 1999).

The model also predicted that a colony could recover from even a very large population of DWV-transmitting mites so long as the mites are removed early enough during the season to prevent excessive infection of the overwintering bees (Fig. 4). For APV a much higher mite threshold would be predicted (Fig. 7) because the acute nature of this virus means that it is, to a large extent, self-limiting. These model predictions are currently being tested by field experiments.

ROLE OF MITE MOVEMENT IN TRANSMISSION OF DWV AND APV

In the current model the rate of viral transmission is mainly dependent on the movement of the mites (vector) between the honeybees (host). This type of indirectly transmitted (i.e. vectored) infection is often referred to as 'frequency-dependent transmission' because the ability of a mite to find a new host is independent of the host density, due to the crowded nature of bees within a colony. This situation is similar to many other infections vectored by biting arthropods, e.g. malaria, where the number of bites (movements between hosts) is fixed and independent of the host density (May & Anderson 1979). In all previous simulations, mites were only allowed to move off their host shortly after it emerged (one move) (Kuenen & Calderone 1997) or from a dying host (Bowen-Walker & Gunn 1998). This will effectively increase the number of infected adults in the colony because the mite is able to transmit the virus to its new host. Preliminary results indicate that a mite feeds on an adult bee once every 4 days (DeD'Aubeterre *et al.* 1999). With a mean phoretic period of 4–6 days (Martin 1998), one horizontal move between adult worker bees between each reproductive phase is a reasonable assumption. Under these conditions the model predicts that the number of DWV-infected adult workers will always be greater than the number of infested adults, and the infected adults will carry the DWV through the winter (Fig. 8). If the mites are allowed to move between two or three adult bees, this results in a corresponding increase in the number of bees carrying DWV (Fig. 8). With APV, the number of infested adult workers and sealed brood cells will equal the number of APV-transmitting mites, and the ability of the mite to move off the dying host becomes important in the speed of colony collapse. It will also be difficult for virulent viruses such as APV to persist in the overt form for long periods of time, such as over the winter. Again these findings are currently being investigated by field experiments. The viral transmission rates used in this model have been simplified pending new information, because the potential complex interactions between the bee–mite–virus system, particularly at low infestation levels, or which include direct transmission of APV between the bees, are the subject of current research.

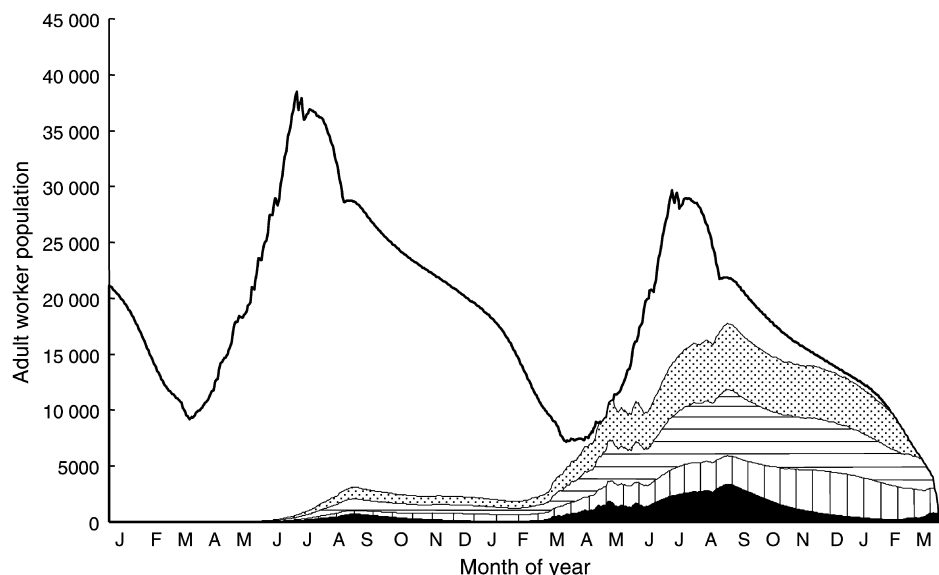


Fig. 8. The effect of mite movement between adult bees on the number of DWV-carrying bees. A colony, as in Fig. 4, was infected with seven mites at the start of the simulation and allowed to develop unhindered. The numbers of DWV-carrying adults when mites remain on their host until it dies (black), move once (vertical lines), twice (horizontal lines) or three (dots) times to uninfected (clear) adult bees are shown.

Conclusions

This model has combined bee and mite population dynamics with viral epidemiology, to produce results that correspond well with field observations and give a clear explanation of how *Varroa*, in association with certain naturally occurring bee viruses, can cause the collapse of a honeybee colony. This study also provides an explanation for why, in *Varroa*-infested colonies, an acute viral pathogen (APV) is quite rare while a chronic pathogen (DWV) has become widely established. One advantage of this model is that it can easily be adapted to investigate other honeybee diseases, either acute or chronic, because any type of survivorship curve can be incorporated directly into the model. In addition, the modular approach allows more subtle effects of a disease to be investigated, such as changes in age structure, which would otherwise be difficult to detect or predict.

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Appendix

Model parameters and equations.

Parameter	Equation or value	Reference
Bee model		
Number of eggs laid by queen, E_t	$= DD L_t N_t P_t$ where	1
dd = degree-days, °C	$DD = -0.0006(dd)^2 + 0.05(dd) + 0.021$	
l_t = day length, h	$L_t = -0.00743(l_t)^3 + 0.312(l_t)^2 - 4.04(l_t) + 16.58$	
n_t = worker population	$N_t = [\log(n_t \times 0.001) + 1] \times 0.672$	
P_t = maximum number of eggs laid	$P_t = 1500$	
Proportion of drone eggs, Z_t	$= E_t([\log(l_t \times 0.1)] \times 0.284) \times ([\log(n_t \times 0.0006)] \times 0.797)$	1
Number of drone eggs laid	$Z_t \times E_t$	1
Number of worker eggs laid	$(Z_t - 1) \times E_t$	1
Worker brood developmental time	492 h	2
Drone brood developmental time	576 h	3
Worker brood post-capping time	282 h	2
Drone brood post-capping time	360 h	3
Natural adult bee survivorship	Variable (see Fig. 2b)	4,5
Natural brood survivorship (%)	$n_t > 9000$ $5000 > n_t < 9000$ $n_t < 5000$	6,7
Egg	94 $0.0049 \times n_t + 50.375$ $0.015 \times n_t$	
Larva	91.7 $0.0042 \times n_t + 54.125$ $0.015 \times n_t$	
Sealed brood	98.5 $0.0021 \times n_t + 79.375$ $0.018 \times n_t$	
Mite model		
Number of viable female mite offspring produced per reproductive cycle	1.01 (worker cells) 2.91 (drones cells)	8
Daily rate of phoretic mite mortality	0.002 (winter) 0.006 (summer)	8
Emergent mite mortality	30% (worker cells) 20% (drone cells)	8
Number of invading mites, M_t	$= \text{number of phoretic mites} \times [1 - \exp(-(r_d + r_w))]$	9
B = weight of adult bees, g	$r_d = (6.49 \times \text{number of available drone cells})/B$ $r_w = (0.56 \times \text{number of available worker cells})/B$	
Virus model		
Vectoring efficiency		10,11
Mite to bee pupae	100% (APV) 89% (DWV)	
Mite to adult bee	100% (APV) 100% (DWV)	
Mite to its offspring	0% (APV) 100% (DWV)	
Infected bee pupae survivorship	100% (APV) 20% (DWV)	10
Infected adult bee survivorship	8 days (APV) Variable (DWV) see Fig. 2b	12,10

¹DeGrandi-Hoffman *et al.* (1989); ²Martin (1994); ³Martin (1995); ⁴Fukuda & Sekiguchi (1966); ⁵Free & Spencer-Booth (1959); ⁶Fukuda & Sakagami (1968); ⁷Harbo (1986); ⁸Martin (1998); ⁹Calis, Fries & Ryrie (1999); ¹⁰S. Martin, B. Ball & N. Carreck, unpublished data; ¹¹Nordstrom (2000); ¹²Bailey (1965).