Modelling biocontrol of *Varroa destructor* using a benign haplotype as a competitive antagonist

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Abstract: The two haplotypes of *Varroa destructor* that have been identified as parasites of the Western honeybee (*Apis mellifera* L.) show disparate levels of virulence towards honeybee colonies. The Korea haplotype has been associated with severe colony mortality, whereas untreated colonies of European *A. mellifera* have survived long-term infestation by the Japan haplotype. The possible existence of a benign haplotype of *V. destructor* raises the prospect that it be used to "inoculate" colonies to provide biocontrol of the virulent haplotype. The feasibility of such a strategy was investigated using a mathematical model. Competition for resources during reproduction is known to reduce varroa mites' reproduction rates as their infestation levels increase. Results from modelling suggested this density-dependent effect is sufficient for an established benign population to prevent the virulent population reaching destructive levels if a colony is subject to sporadic influxes of virulent mites. A colony faced with a continuous influx of mites could be protected if the proportion of virulent mites in the influx were below a threshold level (dependent on length of breeding season and intensity of influx). This condition might be achieved by "inoculating" neighbouring apiaries and controlling feral colonies in the vicinity. Decreased brood cell invasion rate by the benign haplotype decreased the threshold level. Any reproductive isolation between the benign and virulent haplotypes would cause further reproductive suppression, driving sporadic influxes of the virulent haplotype to extinction and conferring greater tolerance to a colony faced with a virulent influx. Increased colony resistance to varroa in the model was synergistic with the inoculation of colonies in the absence of reproductive isolation, but potentially antagonistic in its presence—although not to an extent that would preclude their joint use.

Keywords: *Apis mellifera*; biocontrol; honeybees; model; *Varroa destructor*; varroa haplotypes

Introduction

The emergence of varroa (*Varroa destructor* Anderson and Trueman) as a parasite of the Western honeybee (*Apis mellifera* L.), having transferred from the Eastern honeybee (*Apis cerana* Fabricius), has resulted in significant impact on honey bee populations and beekeeping in a number of countries (Martin, 1998; Sammataro *et al*., 2000).

Varroa destructor, recently distinguished from *V. jacobsoni* Oudemans, parasitises *A. cerana* in mainland Asia. Two of the six identified haplotypes of *V. destructor*, the Korea (Russian) and Japan (Thailand) haplotypes, have become pests of *A. mellifera* (Anderson, 2000; Anderson and Trueman, 2000). While *A. cerana* has a number of behavioural adaptations that confer resistance to varroa (Peng *et al.*, 1987), European *A. mellifera* has little or no resistance to *V. destructor* (de Guzman and Rinderer, 1999).

The transfer of *V. destructor* from *A. cerana* to *A. mellifera* likely first occurred in Far-Eastern Russia (Danka *et al.,* 1995) and the earliest records of colony losses in *A. mellifera* due to varroa were in East Russia and China in the 1960s (Smirnov, 1978). Subsequently, *V. destructor* has spread to most areas where *A. mellifera* is kept (Martin, 1998), and has caused the loss of huge numbers of *A. mellifera* colonies in many countries (de Guzman and Rinderer, 1999), often necessitating regular treatment to control mite numbers.

The degree to which *V. destructor* affects *A. mellifera* varies according to a number of factors including the subspecies of *mellifera*, bee brood-rearing patterns, climate (Kraus and Page, 1995; Rath 1999; Sammataro *et al*., 2000) and the haplotype of *V. destructor* (de Guzman and Rinderer, 1999). Global differences in the survival of varroa-infested colonies of European *A. mellifera* colonies have been linked to differences in virulence between the two haplotypes, with evidence that the Japan haplotype may be relatively benign (de Guzman and Rinderer, 1999; Oldroyd, 1999).

Oldroyd (1999) put forward the intriguing

suggestion that if the Japan haplotype were suitably competitive, it might be used in the biocontrol of the virulent Korea haplotype. This paper explores the feasibility of using a benign varroa haplotype as an antagonist to a virulent haplotype and the potential for biocontrol. In this paper, benign is used in the sense of not causing significant damage at a colony level, while acknowledging that benign mites will have an impact on individual bees.

Background biology

Varroa life cycle

Varroa reproduces within capped honeybee brood cells. A female mite enters a cell prior to capping, and 60 hours post-capping lays eggs at 30-hour intervals, with the first typically being a haploid male and the rest being females (Sammataro *et al.*, 2000). Mother and offspring feed on haemolymph from the developing bee (Donzé and Guerin, 1994). Mother and matured female offspring leave the cell with the emerging bee, while the male and immature females perish (Martin, 1998). In light infestations, females usually breed alone in their cells and brother–sister mating occurs between offspring. In heavy infestations, multiple females often breed in the same cell, allowing more diverse mating, but also increasing offspring mortality through increased competition for food (Fuchs and Langenbach, 1989; Martin, 1995). Thus per capita varroa reproduction rate decreases with increased mite density.

Varroa mites outside brood cells are referred to as phoretic and generally live on adult honeybees, feeding on bee haemolymph (Bowen-Walker *et al.*, 1997). Drifting or robbing bees carry phoretic mites between colonies (Sakofski, 1990). Rates of mite influx into colonies vary with season, nectar flow, and colony interaction, and are usually low in spring and high in late summer or autumn (Sakofski *et al.*, 1990; Greatti *et al.*, 1992; Kraus and Page, 1995). Peaks in influx rate per colony varied from 5 per day (Kraus and Page, 1995) to 76 per day (Greatti *et al.*, 1992). Beekeeping practices may exacerbate varroa transfer by increasing robbing and drifting, or through the transfer of bees and brood.

Effects of varroa

Varroa can cause a number of pathologies, collectively referred to as varroasis. These include deformities, increased brood mortality, reduced body weight and gland size, and premature death of adult bees (de Guzman and Rinderer, 1999). Workers from infested brood may exhibit increased drifting and altered foraging behaviour (Goodwin and van Eaton, 2001). Many varroasis symptoms are likely due to secondary viral infections (Ball, 1997; Bowen-Walker *et al.* 1999). Varroa can vector and induce Acute Paralysis Virus (APV), Chronic Paralysis Virus (CPV) and Deformed Wing Virus (DWV) (Ball, 1997; Bowen-Walker *et al.*, 1999). DWV may even replicate within varroa, thus increasing the probability of pathology (Bowen-Walker *et al.*, 1999).

A large varroa population may cause its host colony to die. This is not a direct function of mite number, and likely depends on the virus being vectored (Martin, 2001). Using a model of viral spread vectored by varroa, Martin (2001) predicted that 10 000 or more phoretic mites vectoring APV would be needed to kill a strong colony, as opposed to between 2000 and 3600 mites vectoring DWV.

Japan and Korea haplotypes

The Japan and Korea haplotypes were characterised by differences in mtDNA sequences (Anderson and Trueman, 2000). They will interbreed, but exhibit a post-zygotic reproductive isolation that varies among populations (Solignac *et al.*, 2005).

Huge losses of European *A. mellifera* occurred in Argentina (Rosenkranz, 1999), Europe, Korea, the Philippines and the United States (de Guzman and Rinderer, 1999). The Korea haplotype is exclusive in these areas, except for the United States where it dominates the Japan haplotype in both frequency and distribution (de Guzman *et al.*, 1999). The Japan haplotype appears to be a recent introduction to the United States with only Korea haplotypes found there before 1997 (de Guzman *et al.*, 1999). Conversely in Brazil where only Japan haplotypes were found before the early 1990s, the Korea haplotype appears to be the more recent, but may now predominate in some areas (Garrido *et al*., 2003). Untreated colonies of European honey bees survived heavy, long-term infestation by Japan haplotypes in Brazil (de Jong and Soares, 1997) and Puerto Rico, and have low colony mortality in Japan (de Guzman and Rinderer, 1999). This tolerance appears to be mite-based since varroa-resistant Italian bees from Brazil showed little varroa-resistance when imported into Germany (Corrêa-Marques *et al.*, 2002). Similarly varroa-tolerance developed by European honeybees in Uruguay, after a period of large colony loss up to the early 1980s, is likely to be mite-based (Rosenkranz, 1999). Isolates of varroa taken in Uruguay in 1994 were Korea haplotypes (Anderson and Trueman, 2000). Rosenkranz's (1999) findings are consistent with a reduction in the virulence of the Korea haplotype in Uruguay, or equally consistent with an introduction of a benign Japan haplotype into that country.

The reason for difference in virulence of Japan and Korea haplotypes has not been established. The

two haplotypes appear to have similar reproduction rates (de Jong and Soares, 1997), and certainly temporal changes in mite fertility in parts of Brazil were not congruent with changes in haplotype, but were possibly host-related (Garrido *et al*., 2003). It is possible that a difference in the viral load between the Japan and Korea haplotypes could explain their differing virulence.

Control of varroa

Both synthetic chemicals (pyrethroids such as fluvalinate and flumethrin, and organophosphates such as coumaphos) and organic ones (essential oils such as thymol, or organic acids such as formic, lactic or oxalic acids) are used to control varroa. While they are generally effective they entail issues such as operator risk, chemical residues, and chemical-resistance developing in varroa (Goodwin and van Eaton, 2001). Other control techniques include brood removal (with or without colony manipulation), the use of mesh boards, and heat treatment (Goodwin and van Eaton, 2001).

Regular treatment is needed to avoid varroa populations reaching damaging levels, with frequency and timing depending on environmental and management factors (Goodwin and van Eaton, 2001). There is no consensus on the level at which a virulent mite population will significantly damage a colony. In the absence of good data, Goodwin and van Eaton (2001) suggested that the damage threshold for New Zealand conditions should be taken as 2500 phoretic mites—a conservative value based on overseas findings.

Biocontrol of varroa

Biocontrol of varroa would mitigate the use of chemical or labour-intensive control methods, and would provide a counter to chemical-resistant strains of varroa. No natural predators or parasites of varroa have been identified as suitable biocontrol agents for varroa (Chandler *et al.*, 2001). However, some bacteria (Tsagou *et al.*, 2004) and fungi (Kanga *et al.*, 2002; Peng *et al.*, 2002; Shaw *et al.*, 2002) are pathogenic to *Varroa destructor*. A field trial indicated that the fungus *Metarhizium anisopliae* was as effective in killing varroa as fluvalinate but would require regular application (Kanga *et al.*, 2003). Additionally, efforts are underway to breed varroa-resistant strains of European *A. mellifera* (Danka *et al.*, 1995; Rinderer *et al.*, 1999).

If the Japan haplotype was used as a biocontrol agent of the virulent Korea haplotype it would act as an antagonist by competing for the same niche, and may confer long-term protection. Control would occur through density-dependent suppression of reproduction and possibly the effects of reproductive isolation. A large, established population of the Japan haplotype could reduce the reproductive rate of a smaller Korea population, perhaps to the extent of zero or negative growth. Thus a promising strategy may be to establish large populations of the Japan haplotype in colonies having few or none of the Korea haplotype. A colony so prepared will be referred to as inoculated, and would rely on the Japan haplotype to counter future incursions of the Korea haplotype.

Methods

In this paper, the Japan haplotype is referred to as the benign haplotype, to allow consideration of a yet-to-be discovered haplotype as an alternative biocontrol agent. The Korea haplotype is referred to as the virulent haplotype. It is premised that a colony can be inoculated with a large benign population and sustain this population without serious long-term damage. Mathematical modelling was used to investigate under what conditions the benign population could prevent the virulent haplotypes from reaching a nominal damage threshold of 2500 phoretic mites.

Several models of varroa population growth have been developed. The first, by Fries *et al.* (1994), incorporated a number of life stages of varroa and was refined by Calis *et al.* (1999) in the area of brood-cell invasion. Further advances included modelling the density-dependence of mite reproduction (Martin, 1998), developing a monitoring tool for beekeepers (Martin, 1999), and modelling the effect of viral spread on colony collapse (Martin, 2001). Wilkinson and Smith (2002) drew from earlier work to construct a combined honeybee–varroa model. DeGrandi-Hoffman and Curry (2004) went a further step by including the influx of mites from other colonies.

The above models all have only one mite population. A new model (described in the Appendix) was developed for this paper, based on work by Wilkinson and Smith (2002), but with differences in several areas. Most significantly it has two haplotype populations (benign and virulent) and uses a different functional form for reproduction. The function used for reproduction brings in interaction between haplotypes, both through mite density effects and through reproductive isolation. The level of reproductive isolation was represented by a parameter, κ, that relates an exponential decline in fertility to the ratio of haplotypes present in a cell (Equations A4 and A5). Four levels of reproductive isolation were considered: $\kappa = 0$ (no isolation), or $\kappa =$ 0.01, 0.05 and 0.10 corresponding to reductions of approximately 1%, 5% and 10% in the fecundity of both haplotypes if they are present in equal numbers in the same cell.

Like previous models, the new model uses delay-difference-equations with a daily time step. It includes the numbers of adult workers and brood (both worker and drone) in a colony of European *A. mellifera*, the phoretic populations of both haplotypes, and implicitly the number of mites reproducing in brood cells. Juvenile stages of varroa were not represented. Hybrid mites were not treated as a separate population, but rather it was assumed that the proportion of virulent genes in interbred offspring is proportional to the proportion of virulent mtDNA in the parent mites. This implies that the threat to a colony can still be quantified by the number of mites with virulent mtDNA.

While the genetics of an *A. mellifera* colony have a strong bearing on rates of *V. destructor* mortality (Ruttner and Hänel, 1992) and reproductive success in brood cells (Harris and Harbo, 2000; Martin and Kryger, 2002), there is no evidence of significant differences in these rates between the Japan and Korea haplotypes. Hence, fecundity and mortality were assumed to be the same for both the benign and virulent haplotypes. However, a biologically plausible difference in reproduction rate between the haplotypes was introduced by varying the benign haplotype's cell invasion rate (BCI). In simulations, "normal", "increased" and "decreased" BCI are respectively equal to, 10% higher than, or 10% lower than the virulent haplotype's cell invasion rate.

Two colony brood patterns were used in simulations (Fig. 1). The first, representing a short breeding season, is a close approximation to that used by Wilkinson and Smith (2002), with brood rearing having a brief summer peak and a break in the winter. The second represents a longer breeding season, with a peak of longer duration and a small quantity of brood present even in the winter. These curves are stylised, but are sufficient for the purposes of this model. Model parameters were adjusted to ensure that predicted growth of a single haplotype of *V. destructor* was close to previous predictions in the literature. The long-term population dynamics of the benign haplotype were predicted in the absence of virulent haplotypes, for both normal and varied BCI.

Biocontrol simulations were performed to evaluate the potential for an established population of benign haplotypes to protect an inoculated colony. Biocontrol simulations were started with the colony in equilibrium with a large, stabilised (though seasonally cycling) population of the benign haplotype. The populations of both benign and virulent mites were predicted over time, and biocontrol was considered a success if the phoretic virulent population stayed below the nominal damage threshold of 2500 phoretic mites. Simulations were typically run for 10 to 15 years to allow discussion of the long-term behaviour. However, for clarity, results against time are plotted for only the first few years. Three different scenarios of virulent mite incursion were run, and were repeated for the case of increased colony resistance to varroa. The first scenario simulated the immigration of the virulent haplotype into an area that it had not colonised, but in which colonies were inoculated with the benign haplotype. This was

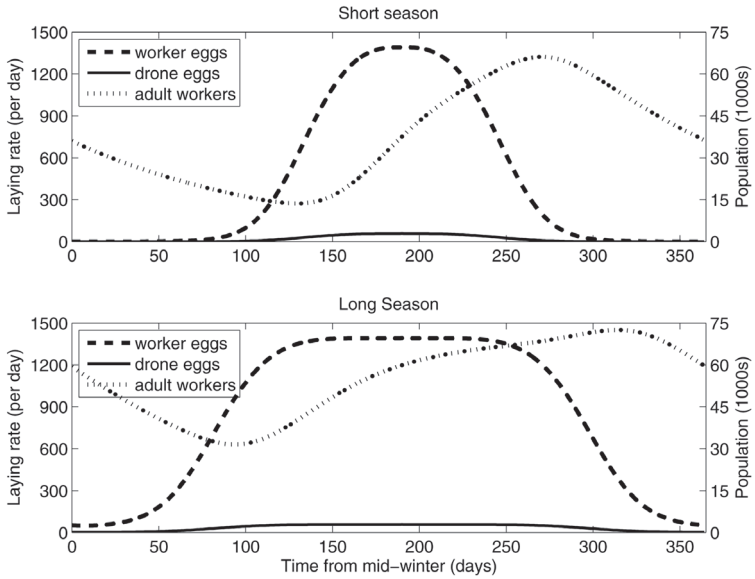

Figure 1. Colony profiles used in the simulations for short and long honeybee breeding seasons. Worker and drone eggs laid per day and the adult worker population are shown over time (measured in days from midwinter).

implemented by seeding an inoculated colony with 1000 virulent mites, 3 months from midwinter, and setting the rate of mite influx to zero.

In the second scenario an inoculated colony is surrounded by colonies infested with only the virulent haplotype, and faces a continual influx of virulent mites. The mite influx patterns used in the model had the same shape as the egg-laying rate (Fig. 1), but was delayed by 2 months so that the peak would occur later in the season. The severity of influx was characterised by the maximum mite influx rate, or "Influx Factor", for which values of 5, 15, 30 and 40 mites per day were used. These respective values correspond to about 580, 1730, 3460 and 4620 mites entering the colony per year for short seasons, and to 1100, 3330, 6620 and 8830 mites per year for long seasons. Greatti *et al.* (1992) found colonies imported from 1874 to 3487 mites per year in an area of high colony density and short breeding season, whereas Kraus and Page (1995) found a colony average of 405 mites in 48 weeks in an area with long seasons. Thus the lower three Influx Factors are likely representative of areas with moderate-to-high mite influx levels, while an Influx Factor of 40 may be considered extreme.

The third scenario involves an apiary of inoculated colonies surrounded by colonies infested by only the virulent haplotype, with an influx of both benign and virulent mites into each inoculated colony. The ratio of virulent to benign mites in the mite influx is expressed in terms of the percent of virulent haplotypes, and is referred to as the virulent loading of the mite influx. The same levels of mite influx were used as in the previous scenario, and the virulent loading varied from 1 to 100% (in steps of 1%). The efficacy of inoculation was expressed in terms of the percent virulent threshold (PVT) of the influx, defined as the maximum virulent loading for biocontrol to be successful.

Worldwide, a number of efforts are ongoing to select strains of European *A. mellifera* that have increased resistance to varroa (Rinderer *et al.*, 1999; Goodwin and van Eaton, 2001). One might expect the effects of an increased resistance of bees to be additive to the effects of colony inoculation with a benign haplotype of varroa. Thus combining the two control methods may be synergistic. Simulations were repeated to predict the additional effect of varroa-resistance, with increased varroa-resistance modelled by a 20% reduction in the number of offspring per breeding female mite. This reduction could arise from a decrease in viable eggs, increased mite mortality in cells, or a reduction in brood post-capping (PC) period. However, accurate implementation of a reduced PC period is limited to reductions by a whole number of days, because the model has a daily time-step and uses PC periods as time delays in variables (see Appendix).

Results

Behaviour of single mite haplotype

The model predictions for the population of a single haplotype of *V. destructor* (setting the numbers of the other haplotype to nought) were similar to the predictions of Wilkinson and Smith (2002). Figure 2 shows the growth of an initial population of 10 benign mites having the same reproduction rates as the virulent haplotype. If the mites do not destroy the colony, their population will eventually be limited by brood availability, and enter into a seasonally driven, periodically varying equilibrium. The proportion of phoretic mites was lower (and therefore overall mite reproduction rate higher) for a long season than for a short season. Thus, in long seasons, mite populations grew faster, and reached a higher maximum earlier. An increase or decrease in BCI had little impact on the long-term periodic equilibrium of the mites' total population (phoretic plus cell invaders) in either season, but affected how quickly it was reached. The maximum relative change for a decreased or increased BCI was respectively a 22% decrease or 23% increase in total mite population after about 1.7 years in short seasons and a 24% decrease or 26% increase after about 1 year in long seasons (Fig. 3). A decreased or increased BCI respectively increased or decreased long-term phoretic populations in the presence of brood, but had no effect in broodless periods.

These simulations did not include mite influx from other colonies, an omission Fries *et al.* (1994) considered reasonable if all colonies in an apiary are uniformly infected.

Biocontrol of sporadic virulent infestations

For normal BCI, an established benign population is likely to prevent a one-off virulent infestation of 1000 mites from reaching destructive levels (Fig. 4). For $\kappa = 0$ the virulent population reached a periodic equilibrium whose maximum was lower in long seasons than short, because the benign population was higher and thus more suppressive. For $\kappa > 0$, the virulent haplotype declined to extinction, with the rate of decline being faster in a long season, and increasing with increased κ (though there was little difference between $\kappa = 0.05$ and $\kappa = 0.10$ in this respect). Increased BCI resulted in a decline in the virulent population, which was accelerated by larger κ and longer seasons. Decreased BCI led to a slower extinction when $\kappa > 0$, but combined with $\kappa = 0$ to allow the virulent haplotype to slowly grow to destructive levels.

Running the simulation with one-off infestation levels of 100 or 10 mites did not change the qualitative behaviour of the responses. Simulations were also run in which the benign population was not established,

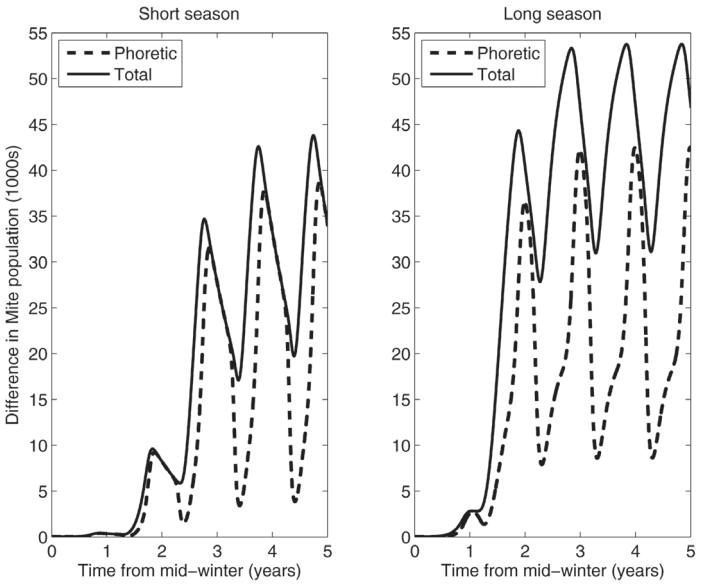

Figure 2. Growth of a population of 10 mites of the benign haplotype if they have the same growth rate as the virulent haplotype, in the absence of an external influx of additional mites from other colonies, corresponds to the brood patterns for short and long bee-breeding seasons. Dashed lines represent the phoretic population and solid lines represent the total mite population (phoretic and those breeding in cells). If the mites do not kill the colony, a periodic equilibrium is reached, with a lower maximum in the short season.

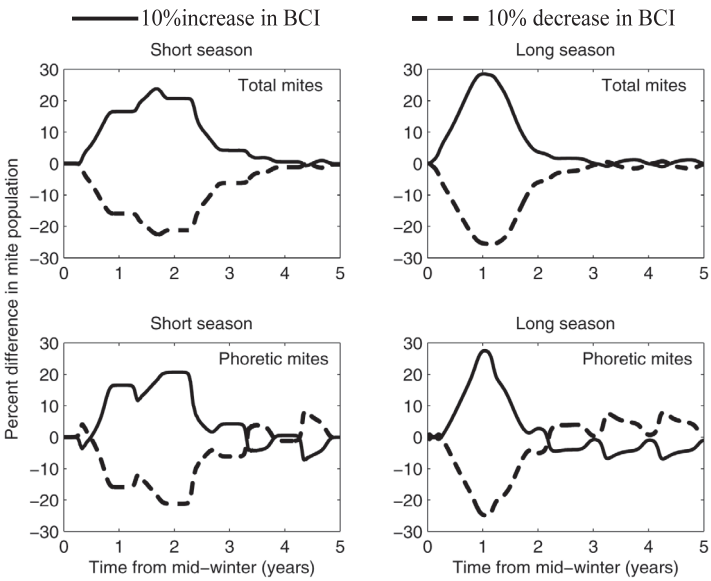

Figure 3. Effect of a 10% increase or decrease in the benign haplotype's cell invasion rate (BCI) on the growth of an initial population of 10 mites, shown as the difference in mite population compared with that for normal cell invasion rates (Fig. 2). A decrease (dashed line) or an increase (solid line) in cell invasion rates respectively decreases or increases mite population growth, but has little impact on the long-term periodic equilibrium of the mites' total population (phoretic plus cell invaders), but has some effect on long-term phoretic population. A longer breeding season reduces the time taken for the curves to converge.

and was comparable to, or less than, the virulent population in number. In these simulations the virulent population exceeded the damage threshold before the benign population could become suppressive, even for increased BCI.

Biocontrol of continuous influx of virulent haplotypes

In an inoculated colony facing a continual influx of virulent mites, the biocontrol success of an established benign population was predicted to strongly depend on the level of virulent mite influx and the level of reproductive isolation, κ, between the haplotypes. For an Influx Factor of 5 per day, biocontrol was successful for $\kappa = 0.01, 0.05$

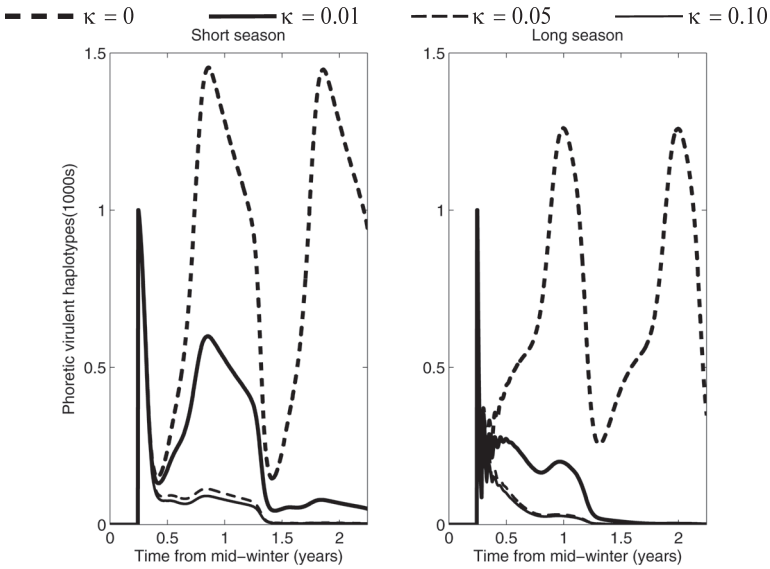

Figure 4. Growth of a one-off infestation of 1000 mites of the virulent haplotype in an inoculated colony when there is no difference between the haplotypes in cell invasion rate. If the reproductive isolation parameter takes the value $\kappa = 0$, the virulent haplotype persists but does not reach destructive levels (>2500 mites), entering into a periodic equilibrium. For $\kappa > 0$, the virulent haplotype will decline to extinction. A longer breeding season accelerates this effect.

and 0.10 (independent of length of season or change in BCI), but not for $\kappa = 0$, except when short seasons were combined with increased BCI. For an Influx Factor of 15 per day, biocontrol was successful for $\kappa = 0.05$ and 0.10 but not for $\kappa = 0$ or 0.01, while for an Influx Factor of 30 per day, biocontrol was successful when $\kappa = 0.10$ and unsuccessful for other values of κ(Fig. 5). Biocontrol was unsuccessful for an Influx Factor of 40 per day for any of the four values of κ. Neither length of season nor changed BCI affected biocontrol success for the highest three mite influx levels.

Biocontrol of continuous influx of benign and virulent haplotypes

The maximum phoretic population of the virulent haplotype, in an inoculated colony, was predicted to increase with increased virulent loading and with increased Influx Factor (Fig. 6), and generally to decrease with increased κ. These responses were qualitatively similar for short and long seasons, and only responses for long seasons are illustrated. PVT, the percent virulent threshold of the influx for which biocontrol is successful, is presented in Table 1. PVT increased (up to a maximum of 100%) with increased κ and decreased with Influx Factor. For long seasons PVT was either lower than, or the same as, for short seasons, with the exception of the case $\kappa = 0.10$ and increased BCI. Compared with normal BCI, PVT was either higher or the same for increased BCI, and either lower or the same for decreased BCI.

Effect of increased honeybee resistance on haplotype interaction

Increased honeybee resistance to varroa resulted in about a 38% decrease in the established benign population, for both long and short seasons, with time taken for an initial infestation of ten mites to reach a periodic equilibrium increasing by about a year.

When an inoculated colony with increased varrroa-resistance faced a one-off infestation of 1000 virulent mites, the response was qualitatively the same as for a non-resistant colony, although the rate of decline or increase was slower.

In the scenario of a constant influx of just virulent mites into an inoculated colony, increased colony resistance resulted in the maximum virulent population being lower when $\kappa = 0$, but higher when $\kappa > 0$, except for the case $\kappa = 0.01$ with an Influx Factor of 40 per day (for which it was lower). Length of season or changed BCI did not affect qualitative behaviour in most instances. Increased colony resistance to varroa did not affect whether or not the benign populations could keep the virulent mite below the damage threshold, except in the case $\kappa = 0.10$ with an Influx Factor of 30 per day for which biocontrol was no longer successful.

With a mixed influx of benign and virulent mites into an inoculated colony, the effect of increased colony resistance on the maximum number of phoretic, virulent mites varied highly non-linearly with κ, Influx Factor and the virulent loading of the influx (Fig. 7). For $\kappa = 0$, increased colony resistance decreased the maximum virulent population, with this decrease being more pronounced for higher virulent loadings and

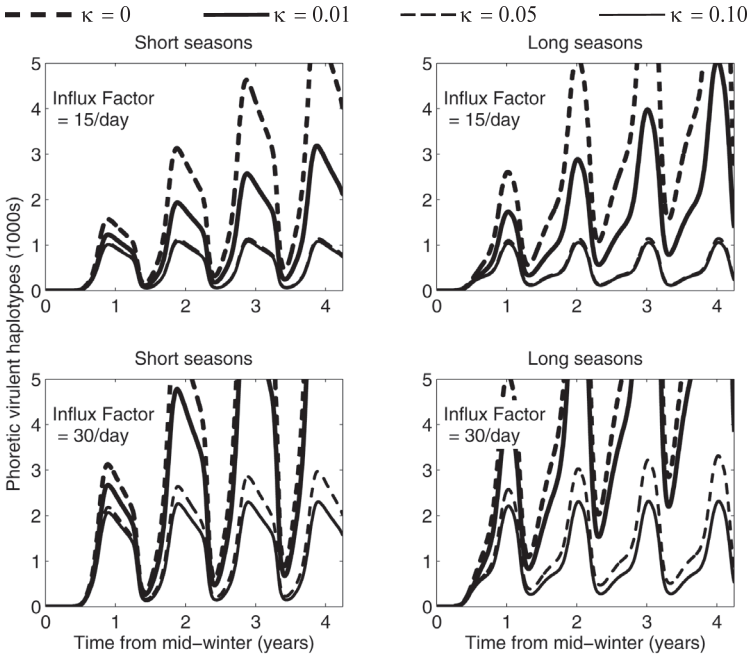

Figure 5. Growth of an initially zero population of the virulent haplotype in an inoculated colony faced with a continuous external influx of purely virulent mites, when there is no difference between the haplotypes in cell invasion rate. For an influx factor of 15 per day, the virulent population was suppressed below the damage threshold (2500 phoretic mites) for values of the reproductive isolation parameter, $\kappa = 0.05$ or $\kappa = 0.10$ but not for $\kappa = 0$ or $\kappa = 0.01$, for both short and long seasons. For an Influx Factor of 30 per day the virulent population was suppressed below the damage threshold for $\kappa = 0.10$ but not for the other values of κ, for both long and short seasons. Where biocontrol did fail, the damage threshold was reached faster in a long season.

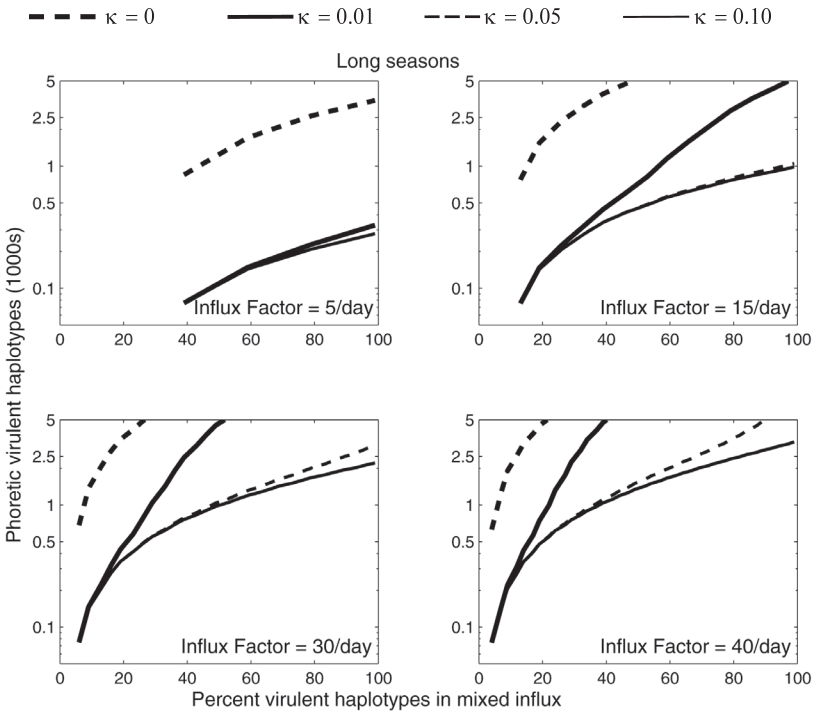

Figure 6. Long-term maximum for phoretic mites of the virulent haplotype in long seasons, when an inoculated colony is faced with an external influx of both virulent and benign haplotypes, when there is no difference between the haplotypes in cell invasion rates. This maximum increases with the percentage of virulent mites in the influx (x-axis) and with the reproductive isolation parameter, κ. Increasing the Influx Factor also increases the maximum virulent population reached.

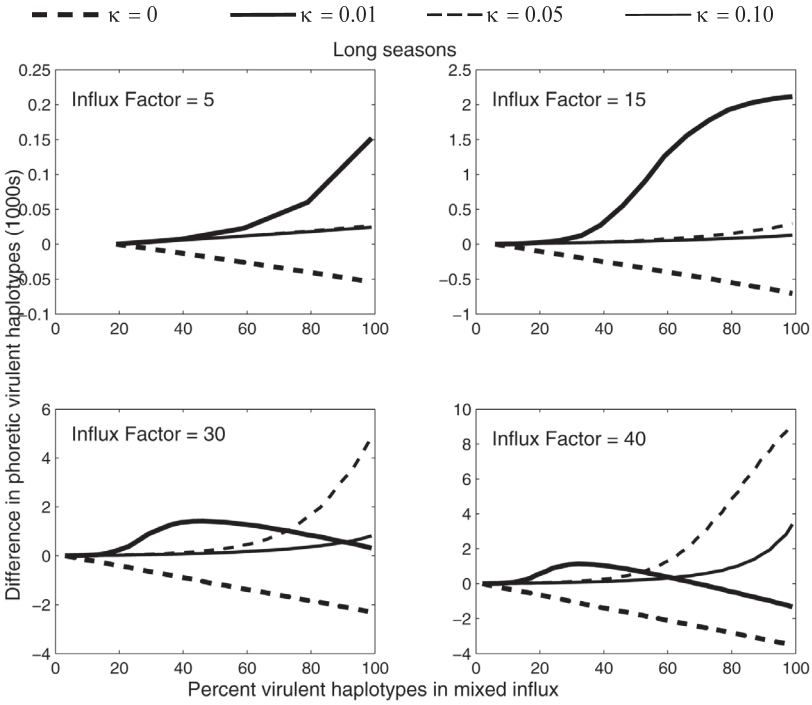

Figure 7. Effect of increased bee resistance on biocontrol using a benign haplotype, expressed in terms of the difference in the maximum long-term phoretic population of the virulent haplotypes, in long seasons, plotted against the percent of virulent mites in the influx. The response varies non-linearly with Influx Factor and value of the reproductive isolation parameter, κ.

Table 1. PVT or maximum percent of virulent haplotypes in a mixed influx for which the virulent haplotype was kept below the damage threshold, in inoculated colony. The intensity of mite influx rate is represented by the Influx Factor. The degree of reproductive isolation between haplotypes is represented by κ. Different rates for the benign haplotype's cell invasion (BCI) were considered: normal, decreased by 10% and increased by 10%.

		Short breeding seasons			Long breeding seasons		
Influx Factor (per day)	κ	Reduced BCI	Normal BCI	Increased BCI	Reduced BCI	Normal BCI	Increased BCI
5	0	99	99	100	59	59	79
	0.01	100	100	100	100	100	100
	0.05	100	100	100	100	100	100
	0.10	100	100	100	100	100	100
15	0	33	39	39	19	26	26
	0.01	79	86	86	66	73	79
	0.05	100	100	100	100	100	100
	0.10	100	100	100	100	100	100
30	0	16	19	23	9	13	16
	0.01	39	43	46	33	39	43
	0.05	86	89	93	83	89	93
	0.10	100	100	100	100	100	100
40	0	14	14	17	9	9	12
	0.01	32	34	34	27	29	32
	0.05	67	69	72	64	67	69
	0.10	79	82	82	79	82	84

Table 2. Difference in PVT (the maximum percent of virulent haplotypes in a mixed influx for which biocontrol is successful) caused by an increased colony-resistance to varroa. Results are shown for different mite influx rates (Influx Factor), different levels of reproductive isolation between haplotypes (κ), and for both long and short breeding seasons. Different rates for the benign haplotype's cell invasion (BCI) were considered: normal, decreased by 10% and increased by 10%. Differences for an Influx Factor of 5 per day were all zero, and hence are not presented.

		Short breeding seasons			Long breeding seasons		
Influx Factor (/day)	κ	Reduced BCI	Normal BCI	Increased BCI	Reduced BCI	Normal BCI	Increased BCI
15	0	0	0	0	0	0	7
	0.01	–20	–20	–13	–13	–14	–13
	0.05	0	0	0	0	0	0
	0.10	0	0	0	0	0	0
30	0	3	0	0	4	3	0
	0.01	–6	–7	–7	–7	–10	–10
	0.05	–20	–20	–20	–20	–20	–20
	0.10	–14	–11	–7	–17	–11	–7
40	0	0	3	0	0	3	2
	0.01	–5	–5	–5	–5	–5	–5
	0.05	–15	–15	–15	–15	–15	–12
	0.10	–12	–13	–13	–15	–15	–15

Influx Factors. For $\kappa > 0$, the difference in virulent population between the increased- and non-resistant cases was slight but positive for low virulent loadings, and (depending on κ and Influx Factor) either grew monotonically, or peaked and declined, as virulent loading was increased. For an Influx Factor of 40 per day the decline after the peak was sufficiently large to make the difference negative for long seasons.

The effect of increased colony resistance on PVT was zero for an Influx Factor of 5 per day (for any combination of κ and BCI) and is presented for other Influx Factors in Table 2. For an Influx Factor of 15 per day, the effect on PVT was zero except when $\kappa = 0.01$ (significant decrease) or when $\kappa = 0$ with increased BCI and long seasons (moderate increase). For Influx Factors of both 30 and 40 per day, the effect on PVT ranged from no to moderate increases when $\kappa = 0$, and from moderate to significant decreases when $\kappa > 0$, with BCI having from no to moderate influence on these results.

Discussion

The simulations suggest that if a benign haplotype of varroa existed, then it could potentially be used as a biocontrol agent to protect a honeybee colony against virulent haplotypes by preventing the virulent haplotype from reaching a nominal damage threshold of 2500 phoretic mites. The mechanisms through which biocontrol could occur are density-dependent reduction in mite reproduction and reproductive isolation. Effective biocontrol would require prior inoculation of a colony with a large, stabilised population of benign haplotypes to confer protection. Oldroyd (1999) had suggested that introducing small numbers of competitive benign haplotypes into a colony infested by virulent haplotypes might offer a means for biocontrol, but the simulation results suggest that this alternative approach would not be successful.

Application of simulated scenarios

The first biocontrol scenario addressed the inoculation of colonies with benign haplotypes to prevent virulent haplotypes becoming establishing in new areas. Results predicted that a large population of benign haplotypes potentially could suppress or eradicate a sporadic infestation of virulent haplotypes if the benign haplotype had an equal or greater reproduction rate, or if reproductive isolation existed between the haplotypes. This raises the issue of whether or not there would be an advantage in introducing genuinely benign haplotypes to the few remaining areas that are varroa-free as a defence against possible future invasion by virulent haplotypes.

The second and third biocontrol scenarios addressed biocontrol in areas already colonised by virulent haplotypes. Results suggested that the potential for biocontrol with a benign haplotype could be most effectively exploited if all colonies in an apiary or groups of adjacent apiaries were inoculated with established populations of benign haplotypes, thus

diluting the influx of virulent mites from feral colonies into managed colonies with benign mites. However, if there was little or no reproductive isolation between haplotypes and mite influx rates were high then biocontrol would be successful only for relatively low percentages of virulent mites in the influx, thus requiring a high inoculation rate for colonies in the area. This might be achievable in practice, by culling feral colonies around apiaries. On a related note, mites from inoculated colonies would be transmitted to feral colonies, and this together with the death of feral colonies infested by virulent haplotypes may cause the virulent haplotype to decline over time, decreasing the threat to managed colonies.

Increased colony resistance

Surprisingly, simulations indicated that breeding for increased varroa-resistance in bees was not necessarily synergistic with using a benign haplotype as a biocontrol agent of the virulent haplotype. The two control methods would be synergistic in the absence of reproductive isolation between the two haplotypes, but could potentially be antagonistic in its presence (though not to the extent that would preclude joint use). This divergence arises because the bees' increased varroa-resistance, on the one hand, reduces the reproduction rate of the virulent haplotype, but, on the other hand, reduces the suppressive effect of the benign haplotype by reducing its population. The reduced efficacy of the benign population may be under- or over-compensated for by the bees' direct suppression of the virulent population, depending on whether or not there is reproductive isolation between the haplotypes.

Feasibility of proposed biocontrol method

In the simulations the population of phoretic benign mites peaked at respectively 39 000 mites and 42 000 mites in a (large) colony with a peak of respectively 66 000 and 73 000 bees for respectively short and long breeding seasons. This number of mites is much larger than the virulent haplotype's nominal damage threshold of 2500 phoretic mites. These may appear to be unrealistically large numbers of mites for a colony to support, even if they were benign. However, Italian bees in Brazil were found, in 1991, to have colony infestation rates with an upper quartile greater than 49 mites per 100 bees and yet showed no sign of colony weakness or death, despite many years of untreated infestation (de Jong and Soares, 1997). This statistic corresponds to 32 000 and 36 000 phoretic mites in a colony the size of, respectively, the short-season and long-season simulation colonies. Thus data from the field suggest that a colony may feasibly support very large numbers of benign haplotypes over the long term. Although de Jong and Soares (1997) found lower mite levels in subsequent years, these measurements were taken in different months (April and May) from their 1991 measurements which were taken in November. The observed reduction in mite levels may have had a seasonl bias.

More important is the existence or not of a benign haplotype. Evidence that the Japan haplotype is benign is circumstantial, and this lack of virulence may well be due to the absence of pathogenic honeybee viruses in areas where this haplotype dominates (B. Ball, Rothamsted Research, U.K., pers. comm.). However, it is equally possible that the Japan haplotype is truly benign. A possible explanation for decreased virulence is that the Japan haplotype is less effective at vectoring or inducing honeybee viruses. Differing levels of viral replication in the Japan and Korea haplotypes might possibly be the mechanism for diverging virulence. Such an explanation is consistent with observations that colony death is linked to viruses, and that varroa's consumption of haemolymph has, in itself, no negative effect on otherwise healthy adult bees (see Goodwin and van Eaton, 2001).

On a practical level, establishing populations of the benign haplotype in colonies may be problematic in areas already infested with the virulent haplotype, since chemical or biotechnical control of the virulent haplotype would be required until the benign population was established. Perhaps isolated areas cleared of virulent haplotypes may be used to prepare inoculated colonies for distribution to other areas. Alternatively, the judicious use of a selective chemical control may be feasible in such a situation, if an appropriately chemical-resistant strain of benign haplotype could be found or induced.

Differences between haplotypes

The model uses the same parameter values for the benign and virulent haplotype. This reflects a lack of knowledge about differences between the Japan and Korea haplotypes. Certainly some aspects of mite reproduction and mortality are influenced by the bees' genetics and the environment, and it is possible that these influences dominate any possible biological differences. However, differences in haplotype sensitivity to chemical cues in the brood may result in different brood cell invasion rates and hence different population growth rates. Additionally, a phoretic mite shows preferences when selecting a host bee, based on the age and the duties of that bee (Kuenen and Calderone, 1997). Differences between haplotypes in these preferences for nurse bees versus foragers may result in differences in reproduction and dispersal.

The discovery that post-zygotic isolation occurs between the haplotypes (Solignac, 2005) raises the challenge of quantifying the degree of isolation for

different populations, and identifying the mechanism (e.g. hybrid inviability, hybrid sterility or hybrid breakdown). The effect that interbreeding of haplotypes has on the virulence of their progeny towards honeybees is also of importance.

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Appendix: Model description

While this model is based on that by Wilkinson and Smith (2002), and retains some of their nomenclature, it differs in a number of areas, especially in that it has two mite populations instead of one, has terms for mite immigration into a colony, uses a different function for reproduction rates, and uses different values for brood post-capping (PC) periods. Wilkinson and Smith (2002) had used respective values of 12 and 14 days for the PC period for workers and drones. These values were used in the model to determine the time after mite invasion that the bee emerges and liberates the new generation of mites. Drone and worker cells were both assumed to be invaded on day 8 after the queen has laid eggs in the cells (Wilkinson and Smith, 2002), corresponding to to worker and drone development times of 20 and 22 days respectively in their model. However, worker and drone brood development times are 19–22 and 24–25 days, respectively (Crane, 1990). Thus the new model used nominal PC periods of 13 and 16 days for workers and drones, respectively, to accord with the more conventionally accepted brood development times of 21 and 24 dats, respectively.

If A_t is the number of adult worker bees on day t, then, for day $t + 1$,

$$A_{t+1} = A_t(1-q_t) + W_{t-21}\,, \tag{A1}$$

where q_t and W_t are, respectively, the fraction of bees dying and the number of worker eggs laid on day t (Wilkinson and Smith, 2002). Thus, Eqn. A1 assumes that an adult worker bee takes 21 days to develop from a newly laid egg. Let P_t^{α} be the number of phoretic mites on day t that are of haplotype α (with $\alpha = j,k$ denoting the benign and virulent haplotypes, respectively). The number of phoretic mites of haplotype α on day $t + 1$ is given by

$$P_{t+1}^{\alpha} = P_t^{\alpha} + E_t^{\alpha} - I_t^{\alpha} - G_t^{\alpha} + X_t^{\alpha}\,, \tag{A2}$$

where, on day t, E_t is the number of emergent mites, I_t is the number of mites invading brood cells, G_t is the number of mites lost through death or migration, and X_t is the external influx of mites. The number of emergent mites is given by

$$E_t^{\alpha} = \sigma_w^{\alpha}(I_{w,t-13}^{\alpha} + V_{w,t-13}^{\alpha}) + \sigma_d^{\alpha}(I_{d,t-16}^{\alpha} + V_{d,t-16}^{\alpha})\,, \tag{A3}$$

where σ_w and σ_d are the survival rates of mites emerging from worker and drone cells, respectively; $V_{w,t}$ and $V_{d,t}$ are the number of viable emergent offspring resulting from mites that invaded the worker and drone cells, on day t, respectively. The delay of 13 days for workers and 16 days for drones in Eqn. A3 corresponds to their PC periods. $V_{w,t}$ and $V_{d,t}$ are dependent on the number of mites of both haplotypes entering worker or drone cells on day t, the number of cells available for invasion, and the reproductive rate γ_w (γ_d) of a single mite reproducing alone in a worker (drone) cell. These dependencies are expressed by the following equations, which were developed by assuming a random distribution of invading mites among the brood cells available for invasion (I. Vetharaniam, *unpubl.*).

$$V_{w,t}^{\alpha} = \gamma_w^{\alpha} I_{w,t}^{\alpha}\left[1 + \frac{\exp(-a_w) - 1}{W_{t-8}}\right]^{\left(I_{w,t}^{\alpha}-1\right)}\left[1 + \frac{\exp\left(-a_w - \kappa W_{t-8}/I_{w,t}^{\alpha}\right) - 1}{W_{t-8}}\right]^{\left(I_{w,t}^{\beta}\right)} \tag{A4}$$

$$V_{d,t}^{\alpha} = \gamma_d^{\alpha} I_{d,t}^{\alpha}\left[1 + \frac{\exp(-a_d) - 1}{D_{t-8}}\right]^{\left(I_{d,t}^{\alpha}-1\right)}\left[1 + \frac{\exp\left(-a_d - \kappa D_{t-8}/I_{d,t}^{\alpha}\right) - 1}{D_{t-8}}\right]^{\left(I_{d,t}^{\beta}\right)} \tag{A5}$$

where $\alpha = j$ or k and $\beta \neq \alpha$. a_w and a_d (both ≥ 0) govern the density-dependent reduction in mite fecundity in worker and drone cells, respectively, when multiple infestation of a cell occurs, and $\kappa \geq 0$ represents the level of reproductive isolation between the two haplotypes. In each of (A4) and (A5), both square-bracketed terms are positive but less than unity, and act to reduce the per capita reproduction rate of haplotype α. The first bracketed term is raised to the power of the number of α haplotypes invading brood cells, which brings in the density-dependent reduction due to increased cell invasions by α haplotypes. The second bracketed term has a smaller value than the first if $\kappa > 0$, and is raised to the power of the number of β haplotypes invading brood cells. This brings in reductions due to increased cell invasions by β haplotypes, through both a density and a reproductive-isolation effect. The latter is reduced by increasing the number of invading α haplotypes, which divides κ in the equations. $\kappa = 0$ represents no reproductive isolation, and in that case the two bracketed terms are equal. The fraction in each bracketed term is divided by numbers of brood cells, which reduces the density and reproductive isolation effects as the number of brood cells available for invasion increases.

Invasion rates for drones and workers for haplotype α are given by

$$i_{w,t}^{\alpha} = \varepsilon_w^{\alpha} W_{t-8} / A_t\,, \tag{A6}$$

$$i_{d,t}^{\alpha} = \varepsilon_d^{\alpha} D_{t-8} / A_t\,, \tag{A7}$$

where D_t is the number of drone eggs laid on day t; ε_w^{α} and ε_d^{α} are parameters governing the rates of invasion of worker and drone cells, respectively, by haplotype α. The total number of mites invading worker or drone cells on day t is (following Boot *et al.*, 1995):

$$I_t^{\alpha} = (1 - m_t^{\alpha}) P_t^{\alpha} \left(1 - \exp(-i_{w,t}^{\alpha} - i_{d,t}^{\alpha})\right), \quad \text{(A8)}$$

The number of mites invading worker cells on day t is given by

$$I_{w,t}^{\alpha} = I_t^{\alpha} i_{w,t} / (i_{w,t} + i_{d,t}), \quad \text{(A9)}$$

and the number invading drone cells on day t is

$$I_{d,t}^{\alpha} = I_t^{\alpha} i_{d,t} / (i_{w,t} + i_{d,t}). \quad \text{(A10)}$$

The loss of mites on day t, is given by

$$G_t^{\alpha} = m_t^{\alpha} P_t^{\alpha}, \quad \text{(A11)}$$

where m_t is the mortality and efflux rate of mites.

An "activity" function $Z(t)$ was defined in order to calculate egg-laying rates, mortality rates for workers and varroa, and rate of external mite influx into colonies:

$$Z(t) = 2 \Big/ \left(1 + \exp\left(\Lambda_1 \left|\sin(\pi (t - 190)/365)\right|^{\Lambda_2}\right)\right), \quad \text{(A12)}$$

where Λ_1 and Λ_2 are parameters that govern the shape of the curve. $Z(t)$ was developed empirically to reproduce the brood curves plotted by Wilkinson and Smith (2002), for which they did not supply the formulae. $Z(t)$ takes values between 0 and 1, and has a period of one year. Different values of Λ_1 and Λ_2 were used for short and long breeding seasons. Laying patterns for worker and drone eggs were taken to be proportional to $Z(t)$:

$$W_t = W_{\max} Z(t), \quad \text{(A13)}$$

$$D_t = D_{\max} Z(t), \quad \text{(A14)}$$

where $W_{\max}$ and $D_{\max}$ are respectively the maximum number of worker and drone eggs laid per day. Rates of mite influx for both haplotypes were also assumed proportional to $Z(t)$, but lagged two months:

$$X_t^{\alpha} = X_{\max}^{\alpha} Z(t - 60), \quad \text{(A15)}$$

where $X_{\max}^{\alpha}$ is the maximum mite influx in a day for haplotype α, $\alpha = j,k$. In the model implementation X_t^{α} was rounded to ensure a whole number of influxing mites. Mortality rates were stepped between winter and summer mortality levels using $Z(t)$ for both workers

$$q_t = q_{\text{winter}} + (q_{\text{summer}} - q_{\text{winter}}) Z(t), \quad \text{(A16)}$$

$$G_t^{\alpha} = G_{\text{winter}}^{\alpha} + (G_{\text{summer}}^{\alpha} - G_{\text{winter}}^{\alpha}) Z(t), \quad \text{(A17)}$$

and mites:

where q_{winter}, q_{summer}, $G^{\alpha}_{\text{winter}}$ and $G^{\alpha}_{\text{summer}}$ are winter and summer mortality rates for workers and mites of haplotype α, respectively.

Parameter values used in the simulation are given in Table A1.

Table A1. Parameter values used in the simulation. The same parameter values were assumed for benign and virulent (Japan and Korea) haplotypes, with the exception of parameters governing cell invasion rate, which were allowed to vary by 10% for the benign haplotype.

Symbol	Meaning	Value
Bee parameters		
D_{max}	Maximum drone eggs laid.	58 per day [a]
W_{max}	Maximum worker eggs laid.	1392 per day [a]
q_{winter}	Rate of worker mortality during winter.	0.008 per day [b]
q_{summer}	Rate of worker mortality during summer.	0.02 per day [b]
Λ_1	Shape parameter for activity.	9 (short season) [b] 4 (long season)
Λ_2	Shape parameter for activity.	2.8 (short season) [b] 6 (long season)
Varroa parameters		
a_w	Controls density-dependent reproduction of mites in worker cells.	0.17[c]
a_d	Controls density-dependent reproduction of mites in drone cells	0.14[c]
κ	Level of reproductive isolation between haplotypes.	0, 0.01, 0.05, 0.10
σ_w	Survival rate of mites emerging from worker cells.	0.62 [d]
σ_d	Survival rate of mites emerging from drone cells.	0.78 [d]
γ_w	Number of fertile female mites produced by a lone female mite reproducing in a worker cell.	1.06 [e]
γ_d	Number of fertile female mites produced by a lone female mite reproducing in a drone cell.	2.44 [e]
m_{winter}	Mite mortality during winter.	0.003 per day [e]
m_{summer}	Mite mortality during summer.	0.016 per day [e]
ε_w	Governs mite invasion into worker cells.	4.48 [e] (±10% for benign)
ε_d	Governs mite invasion into drone cells.	51.92 [e] (±10% for benign)

[a] Based on a 4% drone brood (Wilkinson and Smith, 2002) and a maximum daily bee egg-laying rate of 1450.
[b] Chosen to reproduce colony profile given by Wilkinson and Smith (2002).
[c] Based on data from the literature, I. Vetharaniam, unpubl.
[d] Calculated, based on values quoted by Wilkinson and Smith (2002).
[e] Wilkinson and Smith (2002).