Title: Neonics and mites can interfere with extending the bee-colony longevity during wintering

Short title: Bee-brood damage causes abnormal seasonal changes in longevity

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Summary: We proposed a mathematical model that can estimate the apparent longevity of bees using only both the number of adults and capped brood. Seasonal changes in apparent-longevity estimated using field experimental data provide the following interesting findings:

The apparent longevity of mite-free and pesticide-free bee colonies is almost constant after wintering until mid-September, then begins to increase from late September, continues to increase until the end of wintering, reaching a maximum just before the end of wintering, and eventually drops sharply to a low level after the end of wintering. Such normal seasonal changes in apparent longevity were confirmed also in mite-free colonies which ingested sugar syrup (SS) with

pesticides and pollen paste (PP) without pesticides. The above results that the longevity of adult bees begins to increase from late September and the fact that worker bees become adult bees on the 21st day after the oviposition of the queen suggest the need to prevent exposure to bee swarms to mites and pesticides from early September as a measure to prevent failure of wintering of bee colonies.

On the other hand, mite-free colonies which ingested neonicotinoid-containing PP and mite-infested colonies, show abnormal seasonal changes in apparent-longevity that cannot increase since late September towards the end of winter, as if the bees are not aware of the approach of winter.

Due to the fact that pollen is the main food of bee brood (larvae and pupa), that mites parasitize on brood, that neonicotinoids maintain their toxicity for a long time, and that important functions and organs of bees are created during the larval and pupa periods, it is presumed that such abnormal seasonal changes in apparent longevity make it impossible for bees to notice that winter is approaching because biological rhythms are disturbed by damage by neonicotinoid-containing PP and parasitic mites.

One Sentence Summary: Damages to functions and sensors by neonicotinoid pesticides and mites in larval and pupal period, may erase the increase in bee-longevity during wintering.

Key words: seasonal changes in longevity, bee colony, abnormal, overwintering, pollen, neonicotinoid, pesticide, mite, field experiment, larval, pupal, period, damage

Main Text: Lifespan of animal individuals is one of the important indicators for evaluating the activity of animals. In eusocial insects that form colonies such as honeybees, colonies can be treated as super-individuals that behave as if they were a single animal. It can be said that the day age (longevity) of the longest-living group of honeybees in a honeybee colony is representative of the period of activity of the honeybee colony. In other words, the activity period of a honeybee colony is considered to be assessed by longevity which is an indicator of the life of the entire colony, rather than the lifespan of an individual bee.

The authors proposed a mathematical model (***1***) that estimates the longevity of the honeybee colony only from the number of adult bees and the number of capped brood, and named the longevity estimated by the mathematical model "apparent longevity" in order to distinguish it from that measured directly. Using this proposed mathematical model and the number of adult bees and the number of capped brood measured in long-term field experiments, we estimated the seasonal changes in apparent longevity (*1*). It was found that the apparent longevity of control (pesticide-free) colony estimated using long-term field experiments in mid-west Japan increased rapidly during wintering, and that just before the end of wintering, the apparent longevity reached 6 to 10 times longer than that in non-winter seasons (*1*). Such a sudden change in apparent longevity cannot be explained only by the labor loads (*2*) such as foraging tasks (*3*, *4*) and nursing tasks (*5*) that have been said so far. Therefore, the authors have proposed a hypothesis that a biological rhythm control system which is genetically pre-incorporated in a honeybee detects seasonal changes in the environment such as temperature, humidity and sunshine duration and results in causing the seasonal changes in longevity (*1*). In addition, the apparent longevity estimated from long-term field experiment results on Maui without cold winters was quite different from the estimated results in mid-west Japan, and significantly fluctuated, but no seasonal changes were observed. In less seasonal-change areas such as Maui, apparent longevity has been found to be greatly affected by physiological phenomena in honeybees, such as the absence of queens and a decrease in egg production (*6*).

There have been many reports on factors affecting the longevity of honeybees. Neonicotinoid pesticides (neonicotinoids) that appeared at the end of the 20th century are known to adversely affect the longevity of honeybees due to their characteristics (long-persistent, highly-insecticidal, systemic, neurotoxic). For example, neonicotinoids themselves directly shorten the longevity of honeybees (*7*, *8*, *9*), or indirectly shorten their longevity as follows: Neonicotinoids can adversely affect the immunity of queen bees and cause the re-development of the disease (*10*); they disrupt the circadian rhythms and sleep of honeybees and can impair honeybee navigation, time memory and social communication (*11*); acetamiprid adversely affects memory-related properties in honeybees, homing ability and expression levels of memory-related genes (*12*). Neonicotinoids reduce the mating frequency of queens, resulting in a decrease in the genetic diversity of working bees, which can adversely affect colony vitality (*13*); imidacloprid damages the development of the nervous system in areas responsible for both the sense of smell and vision in the larval stage of honeybees, inhibiting the activity of honeybees (*14*).

Moreover, *Varroa* mites also shorten the longevity of honeybees as follows: *Varroa* mites and deformed wing virus (DWV) reduce the lifespan of winter honeybees (*15*); adult bees emerging from

capped brood in the presence of *Varroa* mites cause their deformed wing and significantly reduce their longevity (*16*); external parasitic mites, *Tropilaelaps mercedesae*, reduce the longevity and emergence weight of honeybees (*17*); *Varroa* destructor parasitic disease and DWV infections cause deformities during the development of Africanized bees, inhibiting cellular immunity and significantly reducing the lifespan of adult bees (*18*).

In addition, combination of the chronic exposure to the systemic pesticide (neonicotinoids and fipronil) and the infestation of the *Varroa* mite (*19*) or the microsporidian parasite Nosema ceranae which is deeply involved with the mite (*20–24*) has synergistic and adverse impact on honeybee survival, and results in shortening the longevity of honeybee colony.

Thus, while it has been found that the long-persistent and systemic pesticide (*e.g.* neonicotinoids) and the external parasitic mite (*e.g. Varroa* mites) and/or viruses mediated by those mites reduce the longevity of honeybees, we cannot find papers regarding the impacts of systemic pesticides and/or *Varroa* mites on the seasonal changes in honeybee longevity other than in our study (*6*). Therefore, since it is important to know what gives to seasonal changes in the longevity of bee colonies in beekeeping, this paper clarifies the following points using a mathematical model (*1*) that estimates the longevity of honeybee *Apis mellifera* colonies from only the numbers of adult bees and capped brood obtained from long-term field experiments (*25–28*) conducted in the mid-west of Japan;

(1) the difference of seasonal changes in apparent longevity between the control (pesticide-free) colony where *Varroa* mites are almost absent (hereafter, mite-free CR colony) and that which is infested with *Varroa* mites (hereafter, mite-infested CR colony);

(2) the difference of seasonal changes in apparent longevity between the mite-free colony where a pesticide is administered through sugar syrup (hereafter, mite-free & toxic SS colony) and that where a pesticide is administered through pollen paste (hereafter, mite-free & toxic PP colony);

(3) the difference of seasonal changes in apparent longevity between the mite-free & pesticide-administered colony and the mite-infested & pesticide-administered colony.

Mite-free control (pesticide-free) colony

The mite-free CR colony (2011/2012 CR-1; 2012/2013 CR-1; 2012/2013 CR-3; 2013/2014 CR-1; 2013/2014 CR-2) (*27–29*) shows seasonal changes in apparent longevity that begins to increase around late September, becomes the longest just before the end of overwintering, becomes instantly short up to near low levels in spring just after overwintering, and then stays almost constant at low levels, albeit with small fluctuations (see Supplementary Figs. S7 to S9) (*1*). These seasonal changes in apparent longevity are normal in areas with four seasons. Incidentally, the apparent longevity of the mite-free CR colony which has failed in overwintering changes as in the case of the overwintering-success mite-free CR colony until the colony extinction (Fig. 1A).

These normal seasonal changes cannot be explained by the theories such as workload on honeybees (*3*, *5*, *29*) and nutritional intake (*30–32*) and so on that have been reported previously. For example, the fact that apparent longevity begins to rise around late September, when honeybees are active, contradicts reports that large workload leads to a decline in apparent longevity. In addition, the abrupt change in the apparent longevity before and after the end of overwintering cannot be explained only

by a not-so-abrupt change in the workloads on honeybees. The previous theories cannot explain that the apparent longevity is increasing, even though the overwintering season is not more nutritious than other times, and a queen bee begins to oviposit during overwintering in mid-February, and the workload on honeybees should increase at this time of year.

Mite-free & toxic (pesticide-containing) sugar-syrup colony

The apparent longevity of the mite-free & toxic SS colony (2011/2012 DF 10ppm/syrup; 2012/2013 FT 10ppm/syrup; 2013/2014 DF 0.2ppm/syrup; 2013/2014 CN 0.08ppm/syrup; 2013/2014 FT 1ppm/syrup; 2013/2014 MT 1ppm/syrup) (*25–27*) begins to increase around late September, continues to increase up to the colony extinction towards winter (Fig. 1A) (see Supplementary Fig. S7 to S9), as is the case of the mite-free CR colony.

Such seasonal changes in apparent longevity of the mite-free & toxic SS colony suggest that the intake of sugar syrup containing a pesticide little adversely affects the innate ability to read seasonal changes mentioned-above. However, the intake of sugar syrup containing a pesticide, especially neonicotinoids, weakens the colony, with normal seasonal changes, and often makes the colony fail in overwintering.

In the long-term field experiments as far (*25–27*, *33*), all honeybee colonies that ingested a long-persistent neonicotinoid failed in overwintering, but honeybee colonies having ingested a much shorter-persistent organophosphate such as fenitrothion are less likely to weaken than those having ingested a long-persistent neonicotinoid, and occasionally succeed in overwintering despite ingesting a sufficient amount of the organophosphate (fenitrothion) (2012/203 FT 10ppm/syrup) (*26*)(see Supplementary Fig. S8).

The reason why sugar syrup containing pesticides has almost no adverse effects on seasonal changes in apparent longevity until colony extinction may be because sugar syrup (honey) is an energy source. Since an energy source such as honey (sugar syrup) is mainly used for the activities of honeybees and is not vital to the creation of biological functions and organs necessary to detect and read seasonal change in apparent longevity during the egg, larval and pupal stages, it can be considered that toxic honey containing pesticides may not affect the seasonal change sensing functions so much. In other words, adult bees, in which all biological functions and organs have been made, mainly consume sugar syrup (honey), which is an energy source, so it can be assumed that toxic sugar syrup (honey) does not cause abnormalities in biological functions or organs that detect and read seasonal changes.

Mite-free & toxic (neonicotinoid-containing) pollen-paste colony

The mite-free & toxic PP colony (2011/2012 DF 0.565ppm/pollen) (*25*) shows quite different seasonal changes in apparent longevity from normal ones that are shown by the mite-free CR colony and the mite-free & toxic SS colony. Abnormal seasonal changes in apparent longevity of the mite-free & toxic PP colony are that the apparent longevity does not begin to increase around late September towards winter, remains almost constant, and moreover, make the colony collapse during overwintering because it cannot increase even during overwintering (Fig. 1B) (see Supplementary Fig. S7).

Since pollen is used as a staple diet for the brood (larvae, pupae) of honeybees and as a raw material for royal jellies which are ingested by queen bees and young larvae, toxic pollen with pesticides can be presumed to cause various disorders and dysfunction when it adversely affects honeybees during gene transcription, functional differentiation and organ creation. The long-persistent pesticide with high toxicity and neurotoxicity, such as neonicotinoid, will continue to adversely affect honeybees over a long period of time once it is ingested during the egg, larval and pupal stages.
Therefore, it is presumed that it will cause gene transcription errors or cause some kind of dysfunction (for example, biological rhythm disorder) when functions and organs are created, and, consequently, that it will not become possible to detect the arrival of winter. On the other hand, in the adult stage where functions and organs are already completed, the influences of toxic honey and toxic pollen on the functions and organs of the honeybee will be considered to be limited.

Mite-infested colony

The apparent longevity of the *Varroa*-mite-infested colony (hereafter, mite-infested colony) (2018 CR-1; 2018 CR-2; 2018 CR-3; 2018 DF-2 0.4ppm/pollen) (*28*) also shows the abnormal seasonal changes, and not only does it not increase around late September, but it remains constant or rather shortens towards winter (Fig. 1A) (see Supplementary Fig. S10), as is the case of the mite-free & toxic PP colony (see Supplementary Fig. S7 to S9).
These mite-infested colonies in 2018-Experiment have collapsed immediately just before or after the start of overwintering.
We will infer what can cause such abnormal seasonal changes:
According to the life cycle of the *Varroa* mite, the mite dives into the larval food in the comb cell before capping the comb cell, then oviposits repeatedly, and grows while sucking the body fluids of larvae and pupae (*34*). In the meantime, mites are thought to cause various diseases and dysfunctions due to injury by the mites and vector-borne viruses. The larval and pupal stages are important for the creation of various functions and organs of the honeybee, and it can be thought that the biological clock is destroyed by mites and viruses and the seasonal changes are not recognized due to an attack by them.
Based on the theory that the damage due to *Varroa* mites on bee larvae can cause such abnormal seasonal changes in apparent longevity which does not increase from around late September towards winter, it is suggested to be necessary at least to exterminate *Varroa* mites from early to mid-September, judging from the life cycle of honeybees, for the bee colony to succeed in overwintering.
Now, we will discuss what can cause a slight difference between the mite-free & toxic PP colony (2011/2012-Experimet) and the mite-infested & toxic PP colonies (2018-Experiment). Both of the mite-free & toxic PP colony and the mite-infested colonies show roughly similar seasonal changes of apparent longevity. Though it was reported that the mites of the mite-free & toxic PP colony did not infest the colony according to the observation results of the previous paper (*25*), it cannot be denied that the abnormal seasonal changes might have been caused by *Varroa* mites.
Therefore, to check the infection status of mites, the number of damaged bees by mites in the mite-free & toxic PP colony (2011/2012 DF 0.565ppm/pollen) was counted, using the photographs of all

combs taken in the field experiment conducted from 2011 to 2012, by the same measuring method as reported in the previous paper (*28*). As a result, it turned out to be that the mite-free & toxic PP colony is hardly infested with mites because its mite-prevalence is 5% or less, while that of the mite-infested colony reaches over 90% (Fig. 2).

Incidentally, we will examine the slight difference between the two kinds of toxic PP colonies (2011/2012-Experiment and 2018-Experiment) from a different perspective. We have reasoned that the abnormal seasonal changes in apparent longevity can be caused by pesticides administered via pollen paste and *Varroa* mites parasitic on larvae. The fact that the mite-infested & toxic PP colony has very similar seasonal changes in apparent longevity with the mite-infested CR colony (Fig. 1B) suggests that the administration of pesticides (dinotefuran) to colonies infested with *Varroa* mites shows little additional bad effect. From this, the following can be deducted.

The very similar abnormal seasonal changes could be possibly caused by that the organs and the functions which extend longevity by feeling the approach of winter are almost completely destroyed by *Varroa* mites. In this case, even if the larvae ingest pollen paste containing the pesticide dinotefuran, the toxic pollen paste would little affect adversely the seasonal change detection ability of the sensory organs and functions destroyed by mites.

According to the above facts, toxic sugar syrup containing pesticides does not cause abnormal seasonal changes in apparent longevity but usual seasonal changes in apparent longevity that extends towards winter, though it weakens the colony and accelerates the colony extinction. Both *Varroa* mites and toxic pollen paste, however, do not increase the apparent longevity from late September towards winter as if honeybees could not notice the approach of winter.

We speculate that these seasonal changes in apparent longevity, which cannot be explained by conventional theories, may have been caused by abnormalities or lack of innate ability to respond to subtle environmental changes around bee colonies (temperature, humidity, weather, sunshine hours, etc.) by reading seasonal changes such as certain biological rhythms based on information embedded in genes in advance.

In other words, it is presumed that neonicotinoid-containing pollen paste and mites damage functions and organs or cause genetic miscopying during the larval and pupal periods, when important functions and organs of honeybees are made. These anomalies can cause disturbances in biological rhythms and cause such abnormal apparent longevity changes due to due to not noticing that winter is approaching.

As discussed previously, if the approach of winter is not noticed, the apparent longevity does not extend as in the neonicotinoid-containing pollen paste colonies in the 2011/2012-Experiment approaches, and remains almost constant at low levels of spring and summer levels. However, in the 2018-Experiment, the apparent longevity becomes shorter rather than constant at low levels towards the winter.

Here, the following question arises: "Why does the apparent longevity of mite-affected colonies in 2018-Experiment decrease from late September (Supplementary Fig. S10) instead of being as constant as like in the bee colony where neonicotinoid-containing pollen paste in 2011/2012-Experment (Supplementary Fig. S7) was administered?" This decrease is presumed to occur for the

following reasons: Because mite-prevalence in the 2018-Experiment increased sharply from mid-September (Fig. 2), it can be estimated that with the increase in the mite-prevalence of bees, the mortality of bees also increased towards winter, and as the result the apparent longevity of bee colonies decreased.

From the results so far, the following can be deduced. Worker bees become adult bees about 21 days after laying eggs, and from late September, the apparent longevity of the bee colony begins to extend due to wintering. Therefore, if mites infest the colony after early September or the colony ingests neonicotinoid-containing pollen, the apparent longevity of the colony cannot be extended from late September as usual, so the apparent longevity will be 20~30 days or less, which is almost the same length as spring and autumn, even after wintering. Therefore, judging from the wintering period of a bee colony of 4-5 months, it is clear that the bee colony cannot successfully overwinter if the longevity of the colony is about 20~30 days. This study revealed that in order to prevent bee colonies from the failure in overwintering, it is necessary to prevent exposure to mites or/and neonicotinoid pesticides on bee colonies from early September.

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Author contributions: Y.Y. and T.Y. conceived and designed the study. Y.Y. proposed and made the mathematical model. Y.Y. made programming to estimate the apparent longevity and performed the calculation. T.Y. and Y.Y. analyzed the data. T.Y. designed and conducted field experiments. T.Y. wrote the manuscript draft. Y.Y. and T.Y. contributed to the final manuscript.

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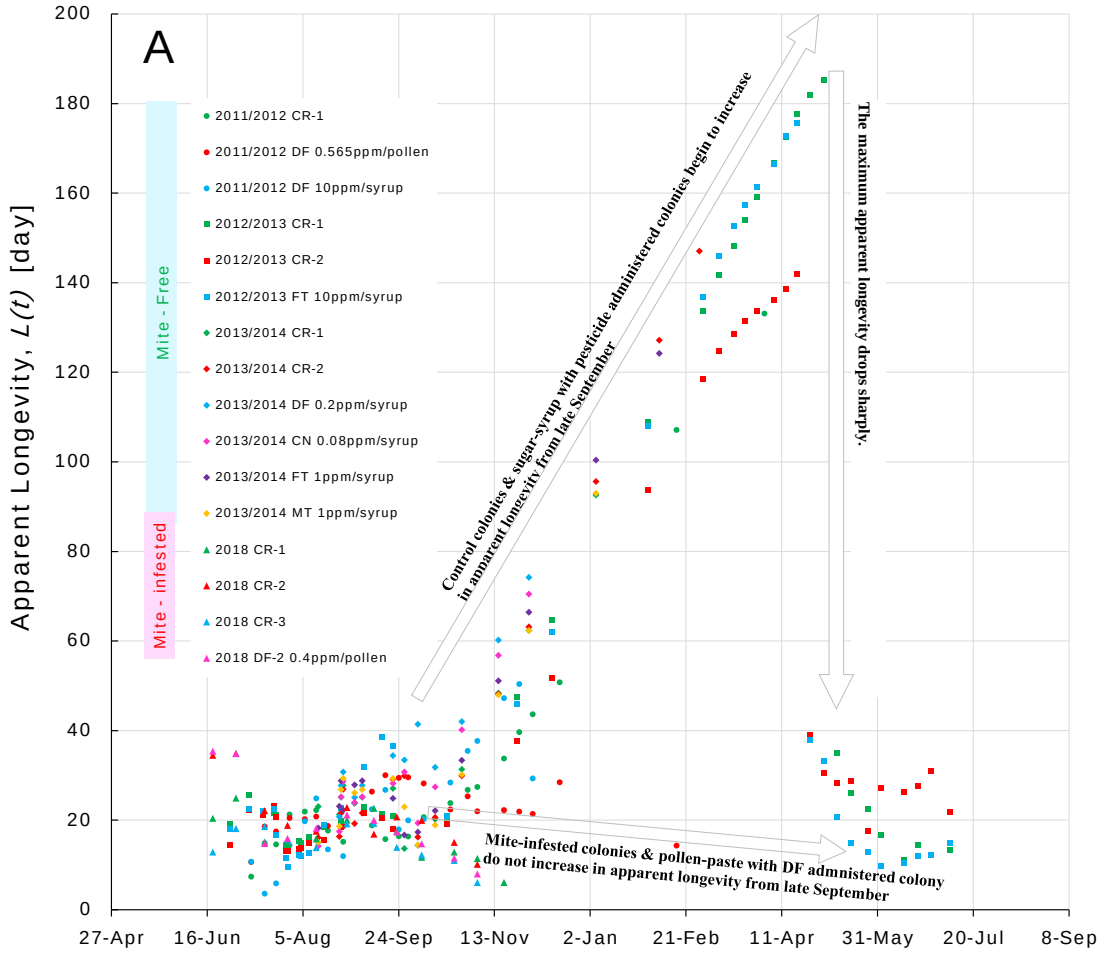
Data and materials availability: Data are available in the supplementary materials.

Supplementary Material

Materials and Methods
Figs. S1 to S10
Table S1 to S7
References (*35–41*)


A
Apparent Longevity, L(t) [day]
200
180
160
140
120
100
80
60
40
20
0
27-Apr
16-Jun
5-Aug
24-Sep
13-Nov
2-Jan
21-Feb
11-Apr
31-May
20-Jul
8-Sep
Mite - Free
Mite - infested
2011/2012 CR-1
2011/2012 DF 0.565ppm/pollen
2011/2012 DF 10ppm/syrup
2012/2013 CR-1
2012/2013 CR-2
2012/2013 FT 10ppm/syrup
2013/2014 CR-1
2013/2014 CR-2
2013/2014 DF 0.2ppm/syrup
2013/2014 CN 0.08ppm/syrup
2013/2014 FT 1ppm/syrup
2013/2014 MT 1ppm/syrup
2018 CR-1
2018 CR-2
2018 CR-3
2018 DF-2 0.4ppm/pollen
Control colonies & sugar-syrup with pesticide administered colonies begin to increase in apparent longevity from late September
The maximum apparent longevity drops sharply.
Mite-infested colonies & pollen-paste with DF admnistered colony do not increase in apparent longevity from late September

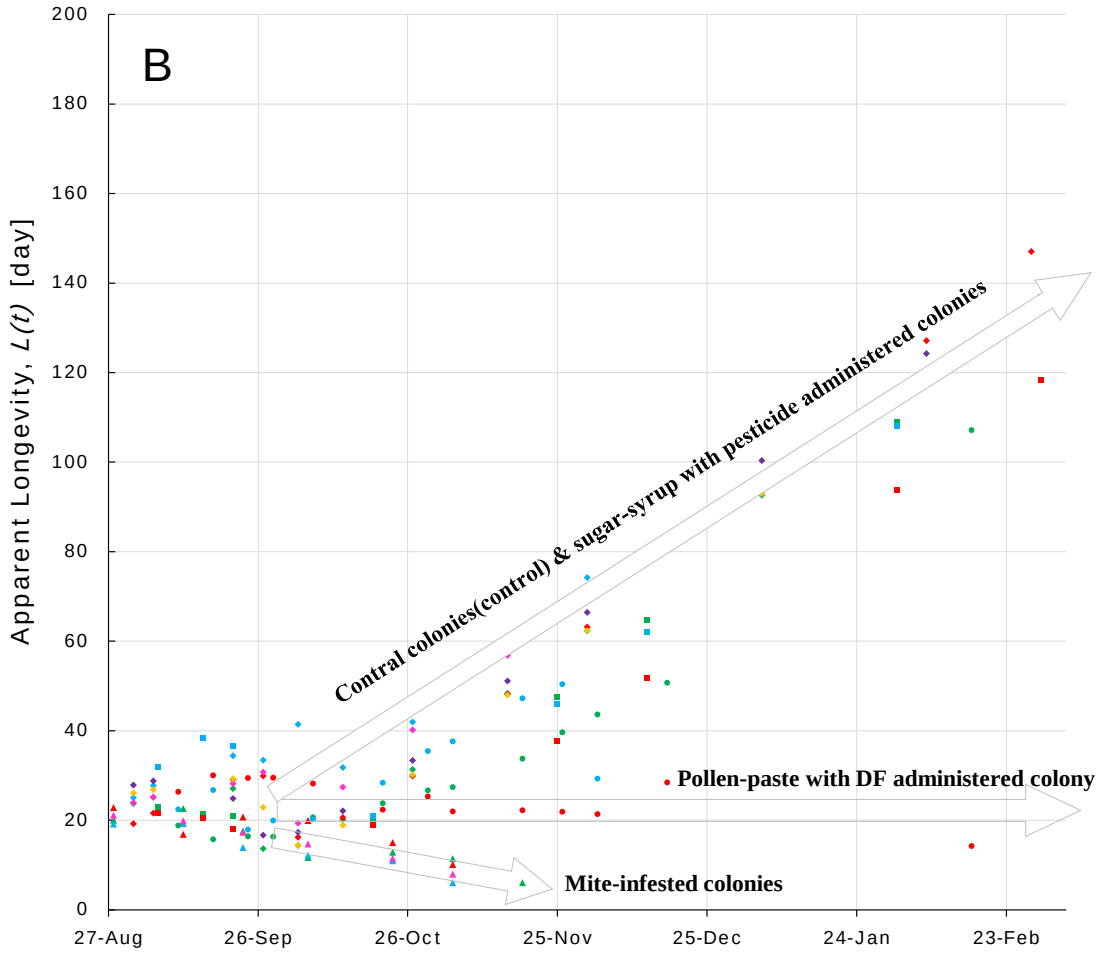

B
Apparent Longevity, L(t) [day]
200
180
160
140
120
100
80
60
40
20
0
27-Aug
26-Sep
26-Oct
25-Nov
25-Dec
24-Jan
23-Feb
Contral colonies(control) & sugar-syrup with pesticide administered colonies
Pollen-paste with DF administered colony
Mite-infested colonies

Fig. 1. Seasonal change in apparent longevities of honeybee colonies in four long-term field experiments.

(**A**): General view. (**B**): Date-axis enlarged view. Apparent longevities were estimated by using the numbers of adult bees and capped brood obtained from four long-term field experiments conducted in : 2011 to 2012 (*25*) for the colonies of "2011/2012 CR-1", "2011/2012 DF 0.565ppm/pollen" and "2011/2012 DF 10ppm/syrup"; 2012 to 2013 (*26*) for the colonies of "2012/2013 CR-1", "2012/2013 CR-2" and "2012/2013 FT 10ppm/syrup"; 2013 to 2014 (*27*) for the colonies of "2013/2014 CR-1", "2013/2014 CR-2", "2013/2014 DF 0.2ppm/syrup", "2013/2014 CN 0.08ppm/syrup", "2013/2014 FT 1ppm/syrup" and "2013/2014 MT 1ppm/syrup"; 2018 (*28*) for the colonies of "2018 CR-1", "2018 CR-2", "2018 CR-3" and "2018 DF-2 0.2ppm/pollen". The colonies which had become extinct before wintering (before late November) are eliminated in this study. Where CR, DF, CN, FT and MT denote control (pesticide-free), dinotefuran, clothianidin, fenitrothion and malathion, respectively. “201x/201y” denotes the experimental period from 201x to 201y, “X Yppm” denotes the concentration “Y ppm” of the pesticide “X”. “syrup” and “pollen” denote sugar syrup and pollen paste of vehicles through which the pesticide is administered into the honeybee colony, respectively. See Supplementary Tables S3 to S6.

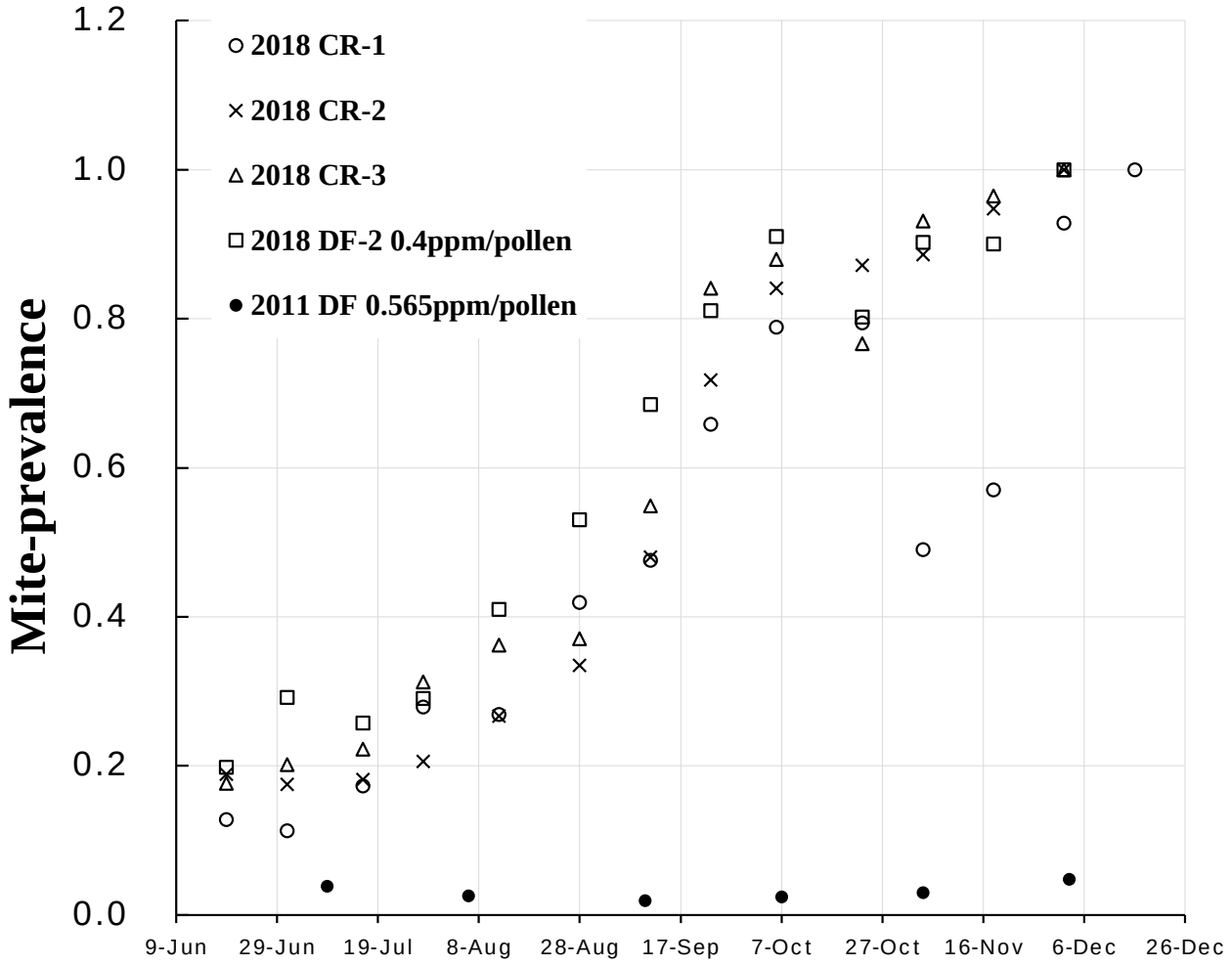


Fig. 2. Comparison of mite-prevalence between the mite-infested colonies and the mite-free & toxic PP colony.

2018 CR-1, 2018 CR-2 & 2018 CR-3: Pesticide-free control colonies in 2018-Experiment (CR-1, CrR-2, CR-3); 2018 DF-1, 2018 DF-2 & 2018 DF-3: Experimental colonies where dinotefuran is administered via pollen paste in 2018-Experiment (DF-1, DF-2, DF-3). 2011/2012 DF: Experimental colonies where dinotefuran is administered via pollen paste in 2011/2012-Experiment.

Mite-prevalence denotes the ratio of bees damaged by mites to total bees in each measurement interval. All colonies in this figure could not begin to increase in apparent longevity around late September. Mite prevalence of all colonies (2018 CR-1, 2018 CR-2, 2018 CR-3, 2018 DF-1, 2018 DF-2, 2018 DF-3) in 2018-Experiment, where dinotefuran (neonicotinoid) were administered via pollen paste, are about 50% in mid-September, about 80% in mid-October, and finally more than 90% (called mite-infested colony). On the other hand, the mite-prevalence of experimental colony in 2012-Experiment (2011/2012 DF) where dinotefuran was administered via pollen paste is almost 2~3%, and at most less than 5%. See Supplementary Table S7.

Supplementary Materials for

Neonics and mites can interfere with extending the bee-colony longevity during wintering

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1 Materials and Methods

1.1 Determination method of apparent longevity

1.1.1 Mathematical model

Using the numbers of adult bees and capped brood obtained from long-term field experiments, the authors have proposed a mathematical model for estimating the apparent longevity of a honeybee colony in each measurement (observation experiment) period of intervals (*1*). There are various day-age groups in the bee group, of which the theory seeks the day-age of the oldest living honeybee group. The following is an overview of the mathematical model

If we can estimate the apparent longevity of a honeybee colony, we can get more comprehensive information from field experiments. The authors have proposed a mathematical model that can estimate the apparent longevity from the numbers of adult bees and capped brood. Here a dynamic parameter called "apparent longevity", *L (t)*, defined in Eq. (S1) is introduced, which depicts the characteristics of the "boundary" day-age where the average honeybee-worker disappears from the bee colony

$$a(t) = \int_{T_{pupa}}^{T_{pupa}+L(t)} u(t-s)p(t-s)ds \qquad \text{(S1)},$$

where *a(t)* and *u(t)* are the number of adult bees and the number of newly capped brood per unit time *t*, respectively. *p (t)* is the eclosion rate from capped brood (pupae sealed on the comb-cells) at time *t*. T_{pupa} is the average period of capped brood, and the period of honeybees *Apis mellifera* is 12 days. If the bee colony is in a steady state, the worker bees die at the same age. *L(t)* represents the longevity of an adult bee. The age distribution of dying bees is given by the narrow Gaussian distribution.

Therefore, it is worth noting that Equation (S1) may provide an estimate of the longevity of a worker bee in a normal bee group.

In order to find the apparent longevity, *L(t)*, in Eq. (S1), it is necessary to know *a(t)*, *u(t)* and *p(t)*. The values of *a(t)* and *u(t)* will be obtained from measurements in field experiments. Since it is difficult to directly measure *u(t)*, it is estimated from Eq. (S2) using the *b(t)* value measured by the field experiment.

$$b(t) = \int_{0}^{T_{pupa}} u(t-s)[p(t-s)]^{s/T_{pupa}} ds \qquad \text{(S2)},$$

where *b (t)* is the number of sealed bee children at time t. Equation (S2) assumes that the pupa is removed as soon as it is dead in the pre-feathering vesicles.

By combining Eq. (S1) and Eq. (S2), it is possible to characterize the honeybee group using a macroscopic amount on honeybees (the number of adult bees and number of capped brood) that are easy to get by experiments. Although *a(t)* in Eq. (S1) and *b(t)* in Eq. (S2) are continuous in the formula (it is a continuous function), it is actually impossible to measure them continuously in field experiments. Since the measurement date is discrete, Equations

(S1) and (S2) were separated under the following assumptions.

1) The day-age distribution of capped brood on a measurement date is assumed to be discretely uniform during the capping period on the comb-cells (12 days), and to be constant between two consecutive measurement days (in each measuring interval). These assumptions are equivalent to that the number of newly capped brood per day, $u(t)$, is constant between two consecutive measurement days. If the number of capped brood measured on a measurement date is expressed in $b(t_k)$, the daily average number of adult bees that newly emerges from the capped brood between one measurement day and the next measurement day is given by $b(t_k)/T_{pupa}$.

2) The eclosion rate is assumed to be constant in two consecutive measurement days.

3) It is assumed that the pesticide intake per larva in two consecutive measurement days is assumed to be the same as the intake of pesticides per bee. In our experiments, it is not possible to directly measure the intake of pesticides per larva in two consecutive measurement days. Therefore, the pesticide intake per measurable adult bee is substituted for that of the larva.

In Eqs. (1) and (2), $a(t)$ and $b(t)$ are known values measured in field experiments, but $L(t)$, $u(t)$ and $p(t)$ are unknown. In two simultaneous equations, one of these three unknown variables must be known before these simultaneous equations can be solved. Fortunately, the eclosion rates, $p(t)$, at various imidacloprid doses has already been reported (*35*). Figure S1 shows the relationship between the imidacloprid dose and the eclosion rate (mean, minimum, maximum value) in a semi-logarithmic graph. The eclosion rate required for calculating the apparent longevity can be obtained using the mean eclosion rate curve in Fig. S1.

Since the pesticide used in this experiment is dinotefuran, not imidacloprid, the dinotefuran concentration must be converted to the imidacloprid dose in order to use Fig. S1. Therefore, the imidacloprid dose equivalent to the insecticidal ability of a certain dinotefuran concentration is estimated as follows.

Field experiment conducted by the authors (*33*) shows that the insecticidal ability of clothianidin for honeybees is about 2.5 times that of dinotefuran (this is the same as the ratio of the recommended insecticidal concentration of dinotefuran for exterminating stink bugs to that of clothianidin).

It can be assumed that imidacloprid's ability to exterminate honeybees is about 2.5 times that of dinotefuran, since the recommended insecticidal concentration of imidacloprid for exterminating stink bugs is approximately the same as that of clothianidin. Pesticide intake per bee in two consecutive measurement days can be estimated from the total pesticide intake and total number of adult bees between the measurement days using the method proposed by the authors (*25*).

In the range of pesticide dosages in previous field experiments (see Table S1), as shown in Fig. S2, the effect on the eclosion rate, $p(t)$, of the pesticide dose is small, and then there is little difference in calculated apparent longevity between when the eclosion rate changes with the pesticide dose per bee for two consecutive measurement days as expressed

by $p(t) = f$ *(pesticide dose)* and when the eclosion rate is 0.9 which is equal to that of the control group. Therefore, in this paper, the apparent longevity was calculated with a constant eclosion rate of 0.9 throughout the experiment as previously reported (*6*). Using the dataset at each measurement date obtained from the field experiment, the apparent longevity, $L(t)$, can be obtained by solving the discrete equation as shown in the previous report (*1*).

1.1.2 Determination procedure

The procedure for calculating the apparent longevity using the proposed mathematical model as described above will be described below. To determine the apparent longevity in the honeybee colony at each measurement (observation experiment), the unknown variables in the equations are the apparent longevity $L(t)$ and the number of brood newly capped on the comb cell $u(t)$. Of these two variables, $u(t)$ can be determined from Eq. (S2). Therefore, the $u(t)$ value determined from Eq. (S2) is substituted to Eq. (S1), and the unknown variable $L(t)$ in Eq. (S1) is determined by an iteration method such as a the Bisection method (*36*), the Simplex method (*37*) and so on, while a certain value $L(t)$ is repeatedly substituted until a difference between the calculated value of the number of adult bees $a(t)$ obtained by the iteration method and the measured value of the number of adult bees by a field experiment reaches a given convergence criterion value (0.0001 in this paper) or less.

In the above calculation to seek the apparent longevity $L(t)$, actual measurement data can be obtained only for each measurement day. Therefore, the apparent longevity $L(t)$ can be sought after the discretization of the expressions while taking account of the life cycle from oviposition to eclosion of the honeybee (egg period 3 days; larval period 6 days; pupa (capped brood) period 12 days).

In this study, the apparent longevity $L(t)$ is determined by the Bisection method (the convergence radius of $L(t)$ is 0.0001 in this work). The calculations were performed using the Ruby programming language (*38*). The flowchart is shown in Figs. S3 and S4. Figure S3 is a whole flow diagram for determining an apparent longevity $L(t)$, and Figure S4 shows a procedure for seeking the apparent longevity $L(t)$ in Eq. (S1) by the Bisection method. These flowcharts are described in a little more detail below.

Here, we will explain the whole flow of the calculation to seek the apparent longevity (Fig. S3). The eclosion rate p_k of a honeybee colony is calculated using the relationship between the dose of imidacloprid per larva and the eclosion rate (Fig. S1) (*35*). Our previous experiments were conducted using pesticides other than imidacloprid, and what can be estimated from experimental data is the intake of a pesticide by an adult bee. Since larvae cannot directly take food (sugar solution, pollen paste) and adult bees (nurse bees) should feed larvae on food, it is assumed that the intake of a pesticide of one larva is the same as that of an adult bee. In addition, it was assumed that the pesticide intake per bee was the same regardless of the role (nursing, forming) in adult bees. That is, "dose of imidacloprid per larva" is equivalent to "intake of imidacloprid per bee".

By the way, imidacloprid and other pesticides (dinotefuran, clothianidin, fenitrothion,

malathion) have different insecticidal ability to bees. With taking into account the insecticidal ability of each pesticide, each pesticide intake is converted into an intake of imidacloprid so that each pesticide can have the insecticidal activity for exterminating the stink bug which is a common pest in Japan (*6*, *26*, *27*).

In a honeybee colony, the total amount of a pesticide ingested at each interval between two consecutive measurement days and the total number of adult bees involved in the pesticide intake at each interval (the sum of the number of adult bees that will be newly born in each interval and the number of adult bees existing from the beginning at each interval) are different at each interval. Therefore, it is necessary to determine the intake of a pesticide per bee for each interval (see below for details). If the intake of a pesticide per bee at each interval is known, the eclosion rate p_k in each interval can be estimated from Fig. S1.

In a honeybee colony, by dividing the sum ($N_k = B_k + a_{k-1}$) of the total number of adult bees (B_k) eclosing between the $(k-1)^{\text{th}}$ measurement date to the kth one and the measured number of adult bees (a_{k-1}) already existing on the $(k-1)^{\text{th}}$ measurement date into the total amount of pesticide intake (TI_k) ingested by the honeybee colony between these two consecutive measurement dates, the average intake of a pesticide per bee ($I_k = TI_k / N_k$) can be determined. In each measurement interval, the intake of a pesticide per bee is converted to that intake of imidacloprid per larva under the assumptions mentioned above, and then the eclosion rate p_k is estimated from Fig. S1. The apparent longevity $L(t)$ calculated with the estimated eclosion rate p_k depending on the pesticide intake differs little from the apparent longevity calculated with the independent eclosion rate of 0.9 (Fig. S2), which is equal to the eclosion rate for the control colony, to the pesticide intake, in the pesticide-intake range in our field experiments. Therefore, the eclosion rate of 0.9 is used to calculate the apparent longevity in this paper.

Next, taking into account the life cycle of the bee (egg period of 3 days; larval period of 6 days; pupa (seal bee child) period of 12 days), the simultaneous equations that discretize Eq. (2) on the kth measurement date is solved, and the number of newly capped brood u_k is determined (**STEP 1**).

The number of newly capped brood u_k determined as described above is substituted for Eq. (S1). Then, while changing the apparent longevity L_k on the kth measurement day with the Bisection method, calculations are repeated till both a difference between the measured number of adult bees and the calculated one and a difference between the old value of L_k and the new one becomes less than 0.0001. When these convergence criteria are satisfied, L_k is the determined value. After iterating the above calculations and completing them for all the number of experiments (k= 1, 2, 3, ⋯ , N) (**STEP 2**), the calculated results are printed.

When determining the apparent longevity L_k by the Bisection method, first L_k is increased with a rough step size (0.5 in this study), and two points (L_k values) where the signs of the Y ($(a_k)_{\text{measured}} - (a_k)_{\text{calculated}}$) are reversed are found (Fig. S4). The two L_k values before and after the sign is reversed are sandwiched, and the step size is further reduced, and the calculations are repeated until both the Y is closest to zero with the Bisection method and the

absolute value of ($L_{k,\ new}$ - $L_{k,\ old}$) becomes less than 0.0001. When the above criteria are satisfied, the value of L_k is regarded as the determined apparent longevity.

1.2 Field experiments

1.2.1 Precautions for obtaining accurate measurements

Both of the numbers of adult bees and capped brood, which are very important for obtaining apparent longevity by use of a mathematical model and can be measured in the experiment, are deeply involved through the life cycle of honeybees each other. If the measurement accuracy of either number is poor, a contradiction will arise between these two measurements. For example, when some of adult bees have gone out foraging, the number of adult bees will underestimate the actual number existing in the honey bee colony. In this case, because there is an unreasonable difference in numbers between the two, apparent longevity cannot be accurately obtained when solving simultaneous equations in a mathematical model, or cannot be sometimes even determined because the simultaneous equations cannot be solved. Therefore, Experiments are conducted while paying attention to the following points to measure accurately the numbers: The experiment is started immediately after dawn to minimize the number of adult bees out of the hive-box. The comb-frame with bees is pulled out from the hive-box as gently as possible, when taking picture, to prevent the bees from flying away, and is gently set on the photo stand. The experiment is conducted while avoiding a windy or rainy day, judging from the weather forecast, because of preventing the bees from becoming restless. In more details, see the previous paper (*28*).

1.2.2 Materials used in the field experiments and their preparation

Dinotefuran (neonicotinoid pesticide) (DF): Starkle Mate® (10% DF; Mitsui Chemicals Aglo, Inc., Tokyo, Japan) was used. Clothianidin (neonicotinoid pesticide) (CN): DANTOTSU® (16% clothianidin; Sumitomo Co. Ltd., Tokyo, Japan) was used. Fenitrothion (organophosphate pesticide) (FT): SUMITHION® emulsion (50% fenitrothion; Sumitomo Co. Ltd., Japan) was used. Malathion (organophosphate pesticide) (MT): MALATHON® emulsion (50% malathion; Sumitomo Co. Ltd., Japan)

Sugar syrup (SS): Granulated sugar was purchased from the Japan Beekeeping Association (http://www.beekeeping.or.jp/) and was composed of 99.7988% purified sugar (granulated sugar), Sodium chloride (salt) 0.1% or more, L-lysine hydrochloride 0.1% or more, and food dye (Blue No. 2) 0.0012% or more). A total of 20 kg of granulated sugar and 13.33 kg of hot water at about 75 ℃ were mixed in a 50 L plastic tank, and then 60% SS of granulated sugar was produced.

Pollen paste (PP): In total, 25 kg of pollen from Spain was purchased from Tawara Apiaries Co., Ltd., Kobe, Japan (https://tawara88.com/about.html). The pollen was used after it was lightly ground with “Kona Ace A-7” flour milling equipment manufactured by Kokkousha Co., Ltd., Nagoya, Japan (http://www.kokkousha.co.jp/). The viscosity of PP was

determined by the ratio of SS in PP, the particle size of the pollen, and the temperature of the PP. The pollen and SS that did not fall when the PP-filled tray was turned upside down was preliminarily examined. As a result, 60% pollen by weight and 40% SS by weight was confirmed to be an appropriate ratio. Pesticide-free PP and PP containing pesticide of desired concentration were prepared in 10 kg each. PP (6 kg of pollen and 4 kg of SS) was prepared in a large plastic bucket by kneading with a drill screwdriver with stirring blades used for high viscosity at a low speed until achieving a uniform paste.

SS with pesticide of a desired concentration: It was produced using 60% SS solution containing the desired concentration of pesticide and sugar. The prepared SS containing pesticide in a 10 L-container was blocked from light using a black bag and stored in a refrigerator.

Preparation and maintenance of the tray filled with PP: The 300 g PP, after being weighed in an upper plate balance scale (accuracy ± 1 g), was packed into a foamed polystyrene tray. Then, the PP tray filled with pollen paste was wrapped to prevent the evaporation of moisture from the tray and stored in a refrigerator to prevent alteration of the PP. The pesticide-free PP tray was stored in the refrigerator compartment, and the PP tray containing a pesticide was stored in the freezer compartment.

1.2.3 Preparation

Field experiments were conducted four times at the resort in mid-west Japan (Shika-machi, Hakui-gun, Ishikawa prefecture, Japan). Three field experiments (*25*–*27*) were conducted at the same location, while the remaining one field experiment (*28*) was conducted at another location nearby. The environment, methods, and publication outlines of these four field experiments are summarized (Table S2). Each hive was arranged at intervals of 80 cm, with its entrance facing south, on a stand of about 20 cm in height, and then a large PVC tray with many perforations of 3mm in diameter to prevent water from collecting was placed between the hive-box and the stand for the measurement of the number of dead bees.

While keeping in mind that the observation results of the photo images are analyzed, various measures were taken to prevent errors and errors in field experiments. For example, in order to be able to infer the experimental contents of the bee colony in the hive, a large display tag was attached at the top of the front of each hive, A seal was attached to the bottom of the hive or on four walls, displaying which part of the hive it was. Moreover, several seals displayed so that the position in the hive of each comb-frame and the front and back of the comb-frame could be seen were attached to the pier of the comb-frame. Further, the name of the hive to be administered was described on a container and a tray of sugar syrup (SS) and pollen paste (PP), in advance, so that the hive where they would be administered to the bee colony was found.

1.2.4 Procedure

The most important data in the field experiment of honeybee colony are both the number of adult bees and capped brood. To obtain these data as accurately as possible, the field experiment was carried out by the following procedure.

STEP-1) To avoid being stung by a bee, dress firmly with protective measures, and conduct experiments.

STEP-2) Experimental tools, recording paper, SS, trays with PP, camera, to prepare the empty hive-box or the like.

STEP-3) Take a picture of the experiment site (entire hive-box) before the experiment (check whether abnormal conditions have occurred).

STEP-4) Count the number of dead bees in a large tray placed under the hive-box while picking up with tweezers. Throw away what you've finished counting out of the tray. It should be noted that the number of dead bees in the hive-box is counted during the internal inspection of the hive-box.

STEP-5) Take a picture of the front of the hive-box before the inspection of the inside of hive-box (record to make sure that the experiment target of the photography is not wrong).

STEP-6) Open the hive-box and remove the cloth that covered the top of the comb-frame of the hive-box, and take a whole photograph of the top of the hive-box. In addition, gently return the bee attached to the cloth in the hive-box.

STEP-7) The tray with PP and the container with SS (tray, feeding frame), which were previously put in the hive-box, are taken out from the hive-box, measuring the remaining amounts of PP and SS by the upper dish balance. Incidentally, when taking out the container from the hive-box, gently return the bee attached to the container in the hive-box.

STEP-8) Gently remove the comb-frame with bees from the hive-box in the order of number (in order from left to right toward the front of the hive-box), take a picture of both sides of each comb-frame. When you are taking a picture, if you find a queen bee, after taking a picture of the queen bee, put it in the queen cage and once isolate it. When finishing the photograph of the comb-frame, put the comb-frame in the spare empty hive-box prepared beforehand, and the lid of the hive-box is closed. The queen bee in the queen cage is placed at the top of comb-frame in the spare hive-box.

STEP-9) After taking pictures of both sides of all the comb-frames with bees in the hive-box, take pictures of the four sides and the bottom in the hive-box in order to determine the number of adult bees left in the hive-box. If you can't find a queen bee when you're taking a picture of a comb-frame, carefully check for the presence or absence of the queen bee, and if present, take a picture. If adult bees are outside the hive-box as well as inside the hive-box (sometimes near the entrance of the hive-box at tropical nights), take a picture to count the number.

STEP-10) If you cannot find the queen bee by all means, check the queen bee on all the comb-frames carefully before taking a picture of capped brood of the next operation.

STEP-11) Take out the comb-frame with bees that had been placed in the spare hive-box in the number order, and return all the bees attached to the comb-frame to the original

hive-box. After taking pictures of both sides of the comb-frame without bee in the order of the comb-frame number, the comb-frame is also returned to the original position of the original hive-box.

STEP-12) When taking a picture of the comb-frame without bee, you may discover some abnormality, such as queen-cells, *Varroa* mites, wax-moth larvae and so on. In this case, keep taking pictures of them. These anomalies are not limited to this situation, and if found, record them in photographs as evidence.

STEP-13) If the queen bee is not found by all means, check in the next experiment. If it is not found next time, by the presence or absence of queen cells and by observing in detail the situation of spawning, etc., the presence or absence of a queen bee is determined.

STEP-14) After covering the cloth on the top of comb-frames, put new SS and PP in the corresponding hive-box, the measurement is ended with the lid of the hive-box.

STEP-15) After completion of the experiment, the test contents are confirmed and described in the research note.

1.3 Counting methods of adult bees, capped brood, mite-damaged bees

Using the photographs taken as described above, it was possible to determine the number of adult bees, the number of capped brood, and the number of mite-damaged bees. Counting these numbers from photographs is an extremely difficult task. Therefore, with the cooperation of Mr. Yoshiki Nagai of Nanosystem Co., Ltd., Kyoto, Japan (http://nanosystem.jp/firm.htm), which develops image processing software, the author has developed computer software in 2012 that automatically counts the relevant numbers from photographic images, and improvements have been made to the software since. The accuracy and operation of the counting have been greatly improved. The counting time and counting accuracy using this software are significantly improved compared to the amount of work required to count by hand directly from the original photo.

However, these counts are still inaccurate because of bee overlap, out-of-focus images, extreme contrast differences in the images, misjudgments of the counting targets, and so on. In this software, it is also possible to manually correct after automatic counting. Therefore, after automatic counting, the counting mistakes due to the automation were corrected by manual operation while zooming into the image, and the corrected number became the final data. Thus, errors still emerge in automation when using this software, but by using manual operation, it is possible to correct the counting errors. The improvements in the counting accuracy and speed under this software contributed greatly to the improvement of the data analysis accuracy and the speed in the field experiments. An overview of this automatic counting system is provided below.

1.3.1. Software developed to assist in accurately counting the numbers of adult bees, capped brood, and mite-damaged bees

This software was developed to count the numbers of adult bees and capped brood accurately. The following explains how to count the number of adult bees present in the image of a comb-frame and how to count the number of capped brood using the image read. These numbers are counted with software using the image from a photo imported into the computer. The number of adult bees in the colony is obtained by summing the numbers of adult bees on both sides of all the comb-frames with the bees and the number of remaining adult bees in the hive-box without the comb-frame. The number of capped brood in a colony can be obtained by summing the numbers of capped brood on both sides of all the comb-frames without bees. The number of adult bees damaged by the *Varroa* mite can be obtained from the same photo used to count the adult bees. How to count bees damaged by *Varroa* mites is described in a separate section. The measurement results obtained by this method, as long as the image remains, can be verified by anyone at any time. Here, the determination procedure of the numbers of adult bees and capped brood and that of the number of mite-damaged bees is described separately. described separately below.

1.3.2 Determination procedure of the numbers of adult bees and capped brood

STEP-1) The image of comb is binarized, and then the difference in brightness between the adult bee or the capped brood in the comb image and the place where there is no bee, and the features of the adult bee ad the capped brood are used to identify the bee and the capped brood. The identified adult bee or capped brood is counted.

STEP-2) In order to count as accurately as possible, before the binarization process of the image, divide the entire image into several areas that seem to have the same threshold value in each area (up to 4 areas) as below. Then, enter the optimal threshold for each divided area (the maximum number of divided areas 4, the split shape is optional). Examples of division: (1) Distinguish between the capped honey area and the capped brood area. (2) Distinguish between areas where there are few bees and areas where bees are dense. (3) Distinguish between different areas of contrast or lightness (e.g., the part where the light hits and the part of the shadow).

STEP-3) Enter the type of key to mark counted adult bees and capped brood one by one (+, *, numbers, etc.) and the color and size of key ((you can change them after the end of the count).

STEP-4) When the count is performed, while automatically marking those counted bee or capped brood in the image, continue counting, the number of counts at that time is displayed at the bottom of the image. After counting the adult bee or capped brood in the image in a short time, the total count in the image is displayed at the bottom of the image. The count status is visually determined, if dissatisfied, by changing the threshold value, recount. In a few tries, even if you change the threshold, if there is no improvement in the count situation, the count of approximate number by automatic measurement in the computer is assumed to have been completed.

STEP-5) After automatic counting by the computer, using the same software, switch to manual count operation, and then correct the count error at the time of automatic counting. Therefore, using a large screen monitor, while further enlarging the image, mark-mistakes of adult bees and capped brood during automatic counting (duplicate count, counting things that are not the object) and forgetting to mark (due to the overlap of bees, the blurred image, the extreme differences in light and dark, etc.) are corrected while visually checking, and all possible efforts are exerted to obtain the number of adult bees or the number of capped brood as accurate as possible (most important and serious work). It should be noted that, even when the software is manual counting, by the removal and grant of the mark, automatically change the number of bees or capped brood. You can resume this fix at any time. If it is determined that the correction is complete, it moves to the measurement of the next new image.

STEP-6) When posting the measured number of adult bees or capped brood in a separate table, call the measured image, check again in the enlarged image, after checking whether there is a count error (correct if you find a mistake), the number is posted to the separate table. The sum total of the numbers of adult bees on all combs and the numbers of adult bees on four walls and bottom in the hive-box, or the sum total of the numbers of capped brood of all combs, is the number of adult bees or the number of capped brood in a bee colony at a certain measurement day, respectively.

1.3.3 Counting procedure of the number of mite-damaged bees

Various sampling methods for *Varroa* mites in a honeybee colony, such as the sticky board method, the roll method, and the powder sugar shake method, have been reported (*39*–*41*). With these methods, it is impossible to determine the total number of *Varroa* mites in a bee colony.

It is presumed that the impacts of *Varroa* mites on a bee colony appear directly on the adult bees with damage caused by the mites. This section aims to measure the total number of bees damaged by mites, not the total number of mites in the bee colony. To count the number of mite-damaged bees as accurately as possible, the images and software used to count the number of adult bees were used again. Using this counting technique for the number of adult bees, it is relatively easy to count *Varroa* mites on an adult bee. On the other hand, it is very difficult to count *Varroa* mites that causes damage to a brood in a comb cell. If any damage is received from *Varroa* mites during the brood stage, there should be a trace of the damage even if the brood becomes an adult bee. An adult bee with traces of its brood stage is considered to be a mite-damaged bee. Moreover, it is also conceivable that the mite dropped out of the adult bee during the bee's development. In this case, it was confirmed whether mite traces remain in the adult bee. Therefore, an adult bee with traces of a mite falling off are also regarded as a mite-damaged bee.

The criteria for the determination of such traces were created based on the information obtained by enlarging the image as is described below. The examples certified to be mite-damaged bees are shown in Fig. S5 (*28*). According to this criterion, using the photographic

image taken for the adult number measurement, it is possible to evaluate the situation of the *Varroa*-mite damage among almost the total number of adult bees in the bee colony. Therefore, the total number of both adult bees with *Varroa* mites and those with traces of *Varroa*-mite damage was determined manually with the software used to measure the number of adult bees while enlarging the adult bee number measurement image. The measurement of the number of mite-damaged bees was a very difficult task and took considerable time (about 6 months). To eliminate the errors between operators, this measurement was carried out by only one person. Figure S6 shows the seasonal changes of mite-damaged bees measured according to the following criteria.

1.3.4 Criteria for determining whether or not it is a mite-damaged bee

In this study, rather than a method of directly counting the number of *Varroa* mites in the bee colony, it is assumed that the number of bees damaged by mites is equivalent to the degree of the bee colony damage caused by mites. The number of mite-damaged bees on the enlarged photographic image of the comb with bees taken to measure the number of adult bees, using the software developed to count the numbers of adult bees and capped brood mentioned above, is manually counted while referring to Fig. S5 according to the following criteria.

In this method, it is not possible to directly evaluate the absolute number of mites in the bee colony, but it is possible to estimate indirectly the damage to the bee colony caused by mites. Additionally, since a photographic image for the measurement of the number of adult bees in a field experiment is used to evaluate the damage to the bee colony caused by *Varroa* mites, it is possible to re-measure the number of mite-damaged bees while checking again and again whenever you need, and it is possible to improve the measurement accuracy. Further, using the photographic image for the past adult number measurement, it is also possible to measure the number of mite-damaged bees during past experiments.

Notes and criteria for determining whether the damaged bee by the *Varroa* mite using the captured image is shown below.

Female *Varroa* mites enter the comb-cell where the larva is just before capping the cell, lay the egg (all male mites) and the number of mites increases in the capped comb-cell. Not only the larvae, it is said to continue to cause damage to adult bees. However, it is impossible to count the number of mites in the capped comb-cell. Therefore, although indirect, there is a trace of mite-damage bees (the presence of mites can be confirmed, there are traces of swelling and inflammation that seem for the bee to have been bitten by mites, wings of the bee are deformed or fallen out, there is a missing mark of the mite on the bee), and the number of adult bees having the above traces can be regarded as an index to estimate the mite-damaged bee in the bee colony. The specific criteria for judgment are shown below.

It is suspected that the three-dimensional one attached to a bee, which is small and round (including the ellipse), is a mite in the color of the red-brown system (including the oval) and red-brown system (including the semi-transparent). A suspected mite is determined whether it

is a mite based on the criteria below and Fig. S5, while being examined from various angles, using an enlarged image. If a suspected mite cannot be determined by all means, it is considered a mite or a trace of the mite.

1) What appears to be planar rather than three-dimensional in the vicinity of the neck is considered to be an innate pattern of a bee appearing when the bee bends its neck or a shadow of other bee wings, etc., and is not considered damage by mites.

2) Small colors other than red-brown and black (especially whitish ones) are regarded as pollen.

3) What the wings appear three-dimensional like wrinkles deformed is regarded as a shadow due to the shrinkage of the wings. However, the cause of the shrinkage of the wings is a large possibility of deformed wing virus mediated by *Varroa* mites. The state of the shrinkage of the wing is scrutinized on the image to determine whether the shrinkage is due to mites.

4) If a bee with shrunk or missing wings has traces of a mite bite, it is considered a mite-damaged bee, even if it is not possible to confirm the presence of a mite.

5) If you can't distinguish between mites and pollen or garbage, you should consider them mites. However, if it is clear that it is other than mites such as pollen and garbage, exclude it.

6) Planar ones are usually determined to be not mites. However, after scrutiny, exceptionally, it may be considered a mite-damaged bee.

7) Except for those that seem to be the rise of muscles, etc.

8) The difference between a mite and garbage is judged by shape, color, surrounding situation, etc., and it counts unnatural things like mites (although it is subjective, counting the one like the mite)

9) Traces bitten by mites (swelling by the bites) are also thought to be damage to bees.

10) Regardless of what appears to be a mite or the number of traces bitten by mites, it is treated as a mite-damaged bee.

11) Traces of a mite fallen out from a bee are treated as a mite-damaged bee.

Just before the larva's cell is capped, the mites lay eggs in the food in the cell, since the eggs grow while sucking the body fluid of the larva hatched in the capped cell, it is almost impossible to find almost a mite even in the open cell. Therefore, it was decided not to evaluate the mites in the cells.

Mites are present in various places in the hive-box, shape is also easy to mistake for garbage and pollen, etc., also present in the invisible place (especially in the comb-cell), although the absolute number of mites is not known, this method to evaluate by the number of adult bees damaged by mites is believed to be a qualitative indicator of the number of presence of mites. Although the number of mites in the cell cannot be known, the infestation state of mites in the bee colony will be reflected in the number of mites adhering to the adult bee.

This method includes an uncertain element, under the above criteria, by measuring in the

same person, and relative comparison of the number of mites between each bee colony, to some extent the time course of each bee group it is considered to represent. By the way, whenever a doubt occurs, again, using the image for the adult bee number measurement taken, recount.

2 Supplementary Figures (Figs.S1 to S10)

Fig. S1. Change in eclosion rate of honeybees with imidacloprid dosage.

Fig. S2. Comparison of apparent longevity between a variable eclosion rate, $p(t)= f$ (dinotefuran-intake per bee), and a constant one, $p(t)= 0.9$.

Fig. S3. Flowchart to find the apparent longevity, $L(t)$: Mainstream scheme.

Fig. S4. Flowchart to find the apparent longevity, $L(t)$: Bisection method.

Fig. S5. Images of mite-damaged bees.

Fig. S6. Number of mite-damaged bees and mite-prevalence.

Fig. S7. Apparent longevity of 2011/2012-Experiment.

Fig. S8. Apparent longevity of 2012/2013-Experiment.

Fig. S9. Apparent longevity of 2013/2014-Experiment.

Fig. S10. Apparent longevity of 2018-Experiment.

Fig. S1. Change in eclosion rate of honeybees with imidacloprid dosage.

The source data on the relation between dose of imidacloprid and eclosion rate of honeybee are as follows (*35*): Eclosion rates are; 89.82±10.10 and 89.17±7.26 % for the control group; the rates are 89.20±4.90 % at the imidacloprid concentration of 0.4 ng/larva, 3.54±4.92 at that of 24 ng/larva, 77.29±6.64% at that of 200 ng/larva, 51.04±6.78 at that of 2000 ng/larva, 30.63±12.31 % at that 4000 ng/larva, 25.21±6.47 % at that of 6000 ng/larva and 8.54±1.72 % at that of 8000 ng/larva, respectively. Since Semi-log graphs cannot handle zero values, the pesticide concentration of the control group was set to 0.001 ng / larva for convenience.

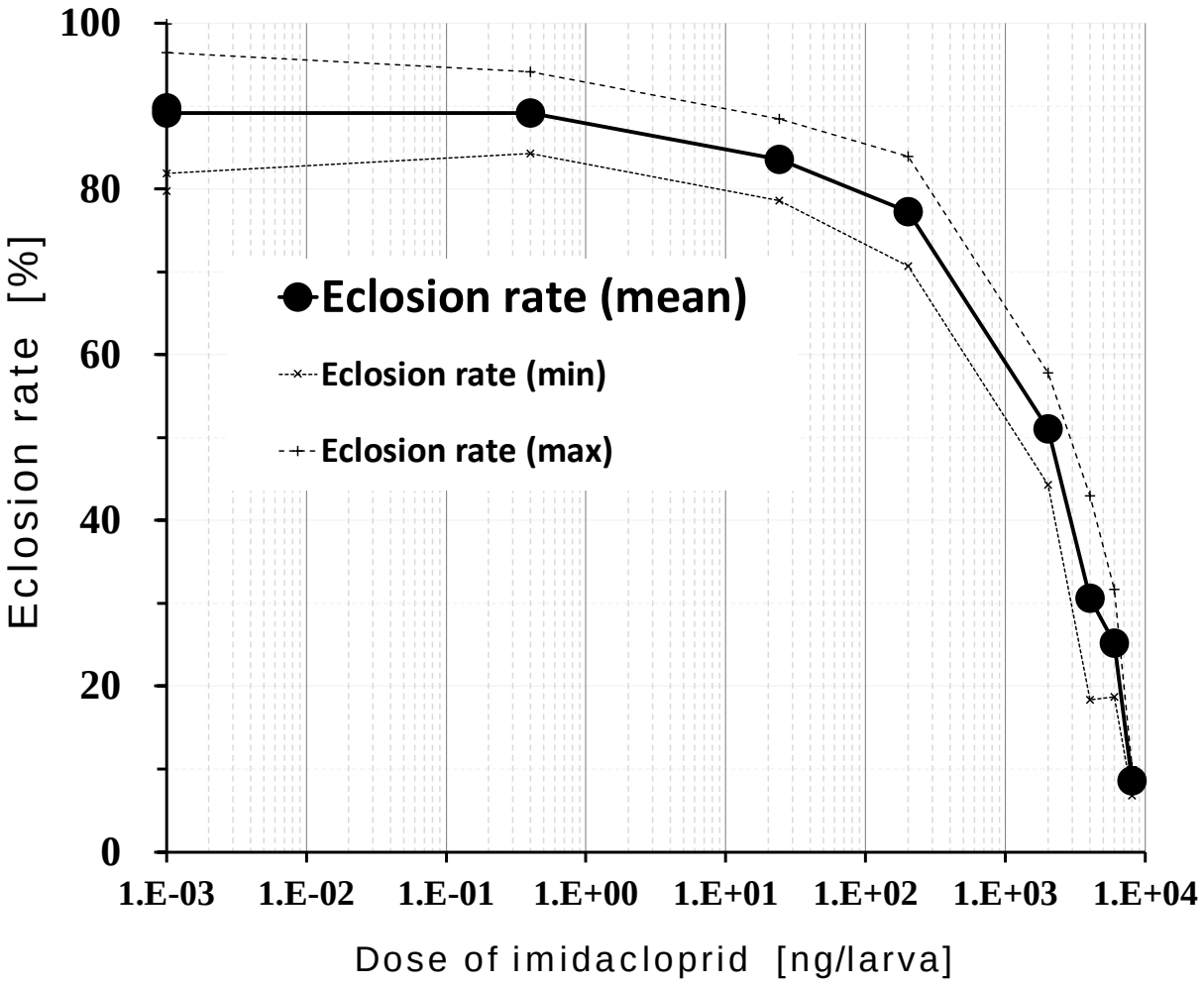

100
80
60
40
20
0
Eclosion rate [%]
Eclosion rate (mean)
Eclosion rate (min)
Eclosion rate (max)
1.E-03
1.E-02
1.E-01
1.E+00
1.E+01
1.E+02
1.E+03
1.E+04
Dose of imidacloprid [ng/larva]

Fig. S2. Comparison of apparent longevity between a variable eclosion rate, *p(t)=f*(dinotefuran-intake per bee), and a constant one, *p(t)*=0.9.

To estimate the apparent longevity of honeybee colony were used the numbers of adult bees and capped brood measured in the field experiment conducted in 2011 to 2012 (*25*). $L(t)_{p(t)=p(t)}$ shows the apparent longevity was calculated with the variable *p(t)* changing with the pesticide intake per bee in a period between two consecutive measurement days; where the ranges of *p(t)* are 0.79–0.9 for the "2011/2012 DF 1ppm/syrup $L(t)_{p(t)=p(t)}$" colony; 0.84–0.9 for the "2011/2012 DF 0.565ppm/pollen $L(t)_{p(t)=p(t)}$" colony, 0.8–0.9 for the "2011/2012 DF 10ppm/syrup $L(t)_{p(t)=p(t)}$" colony, 0.835–0.9 for the "2011/2012 DF 5.65ppm/pollen $L(t)_{p(t)=p(t)}$" colony, respectively. Incidentally, the intake of dinotefuran (DF) by one honeybee in a period between the observation interval was obtained according to the procedures reported in the previous paper (*25*). $L(t)_{p(t)=0.9}$ shows the apparent longevity was calculated with the constant *p(t)* of 0.9.

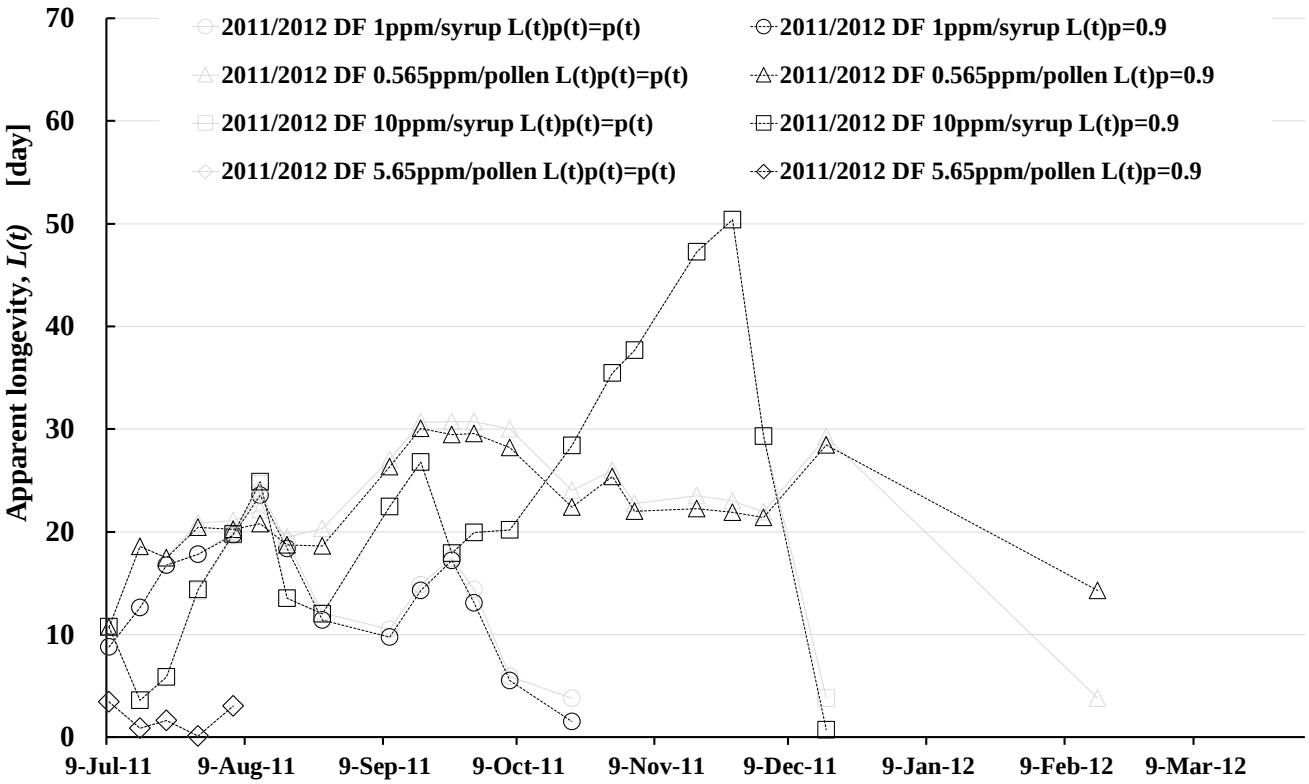

2011/2012 DF 1ppm/syrup L(t)p(t)=p(t)
2011/2012 DF 1ppm/syrup L(t)p=0.9
2011/2012 DF 0.565ppm/pollen L(t)p(t)=p(t)
2011/2012 DF 0.565ppm/pollen L(t)p=0.9
2011/2012 DF 10ppm/syrup L(t)p(t)=p(t)
2011/2012 DF 10ppm/syrup L(t)p=0.9
2011/2012 DF 5.65ppm/pollen L(t)p(t)=p(t)
2011/2012 DF 5.65ppm/pollen L(t)p=0.9
Apparent longevity, L(t) [day]
70
60
50
40
30
20
10
0
9-Jul-11
9-Aug-11
9-Sep-11
9-Oct-11
9-Nov-11
9-Dec-11
9-Jan-12
9-Feb-12
9-Mar-12

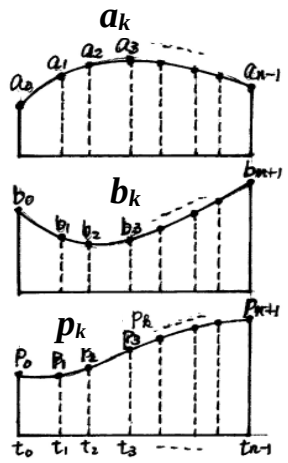
Fig. S3. Flowchart to find the apparent longevity, *L(t)*: Mainstream scheme.

The calculations were carried out using a software written in the Ruby programming language.

START

INPUT THE DATA

t_k **: Elapsed days**
a_k **: Number of adult bees**
b_k **: Number of capped brood**



STEP 1

Discretize Eq. (S2) and solve a set of simultaneous equations in unknown u_k (number of newly-capped brood).

Eq.(S2) $\cdot\cdot\cdot$ $$b_k = \int_0^{Tp} u(tk-s)[p(tk-s)]^{\left(\frac{s}{Tp}\right)} ds$$

$$b_k = \sum_i ui \int_0^{Tp} [pi]^{\frac{s}{Tp}} ds$$

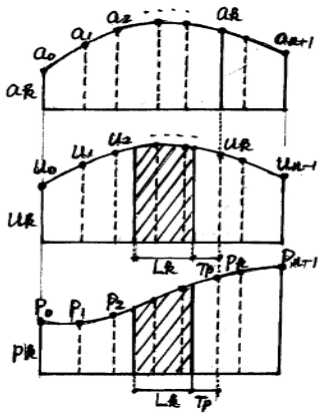
$$b_k = \mathrm{ui} \sum_i \int_0^{Tp} [pi]^{\frac{s}{Tp}} ds$$

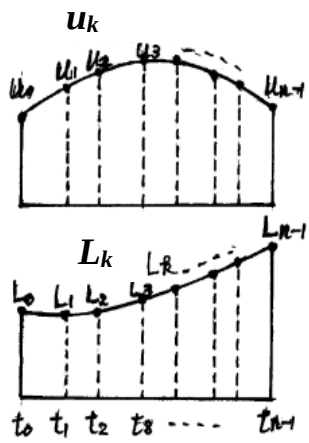
$$\mathrm{b} = [D] \cdot \mathrm{u}$$
$$\mathrm{u} = [D]^{-1} \cdot \mathrm{b}$$

STEP 2

Find unknown L_k in Eq.(S1) using u_k obtained by STEP 1 and input data of a_k and p_k by a bisection method.

Eq.(S1) $\cdot\cdot\cdot$ $$a_k = \int_{Tp}^{Tp+Lk} u(tk-s)p(tk-s)ds$$





PRINT THE DATA

t_k **,** a_k **,** b_k **,** p_k **,** u_k **,** L_k

END

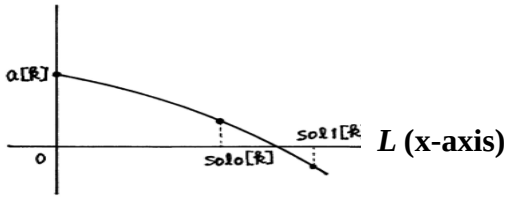
Fig. S4. Flowchart to find the apparent longevity, *L(t)*: Bisection method.

STEP 2
(Bisection method)

Create an evaluation function of Y(L) :

$$Y(L)= a[k]-\int_{Tp}^{Tp+L} u(tk-s)p(tk-s)ds$$

Y(L)



Set convergence criteria:
$x_\varepsilon = 10^{-4}$, $y_\varepsilon = 10^{-4}$

Execute the following process for the number of elements of $a[k]$

Increasing the value of L sequentially from zero in 0.5 increment, find the value of L where Y(L) is negative for the first time. Set the value of L at this time as sol-1[k] and then set previous value of L as sol-0[k].

Execute the following process for the number of elements of sol-1[k]

L_{t0} = sol-0[k], y_0 = Y(L_{t0})
L_{t1} = sol-1[k], y_1 = Y(L_{t1})

$|y_0| < y_\varepsilon$ or $|y_1| < y_\varepsilon$ or $|L_{t0} - L_{t1}| < x_\varepsilon$?

Yes

No

Calculate $L_{t2}= (L_{t0} +L_{t1})/2$ & y_2 = Y(L_{t2}) and then replace y_0 or y_1 with the same sign as y_2 with y_2 to make a new set of y_0 and y_1.

Add L_{t1} to the solution as $L_k = L_{t1}$.

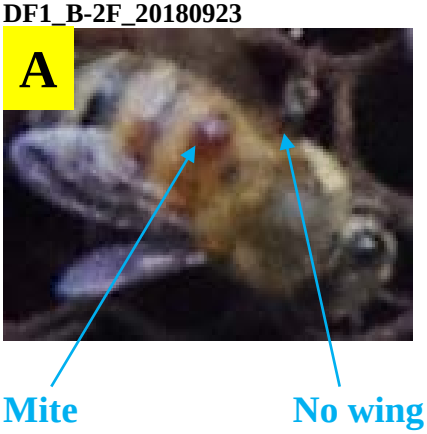
NEXT

Fig. S5. Images of mite-damaged bees.

The above images were obtained from the photographs of adult bees on comb-frame taken in the field experiments. Referring the above, we judged whether the adult bee was damaged by mites while enlarging the image. Several representative images of adult bees that appear to have been damaged by *Varroa* mites are shown. Each image is an example of a mite-damaged bee. **A:** A mite on the back of an adult bee (worker bee) whose wing is removed. **B:** Mites on the bee's leg and back. **C:** A mite on the head of a drone. **D:** The trace of a mite that fell out of the bee's back. **E:** Trace of a mite that fell out and a bee that has no wings. **F:** Traces of mites that fell out of the bee's back and a bee with no wing. **G:** Traces of a mite that fell out and a bee that seems to have been bitten by mites.

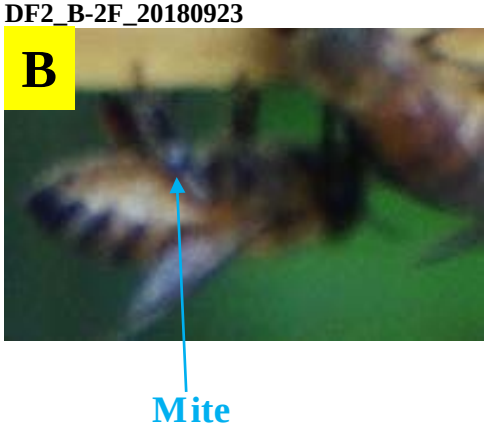
Mite on the bee's back

DF1_B-2F_20180923



Mite on the bee's leg & back

DF2_B-2F_20180923

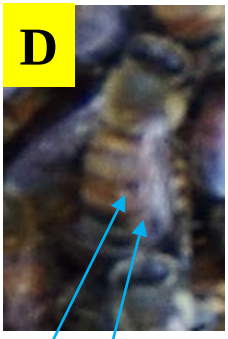


Mite on the head

DF2_B-2B_20180923

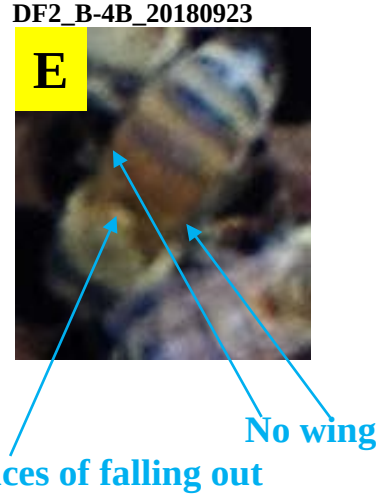
Traces of mites

DF2_B-2B_20180923



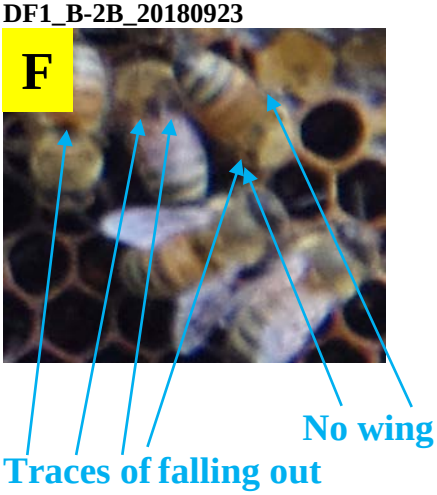
Traces of mites

DF2_B-4B_20180923



Traces of mites

DF1_B-2B_20180923



Traces of mites

DF2_B-2B_20180923a

Fig. S6. Number of mite-damaged bees and mite-prevalence.

Details of these data in the field experiment conducted in 2018 are reported in the previous report (*28*). The mite-prevalence denotes the ratio of the number of damaged bees by *Varroa* mites (mite-damaged bees) to the total number of adult bees in a period between each measurement interval.

Mites/Adults denotes the mite-damaged bees /total bees (mite-prevalence).

2018 CR-1, 2018 CR-2 and 2018 CR-3 denote the control colony which is infested with *Varroa* mites.

2018 DF-1, 2018 DF-2 and 2018 DF-3 denote the colony being infested by *Varroa* mites and to which dinotefuran is administered via pollen paste.

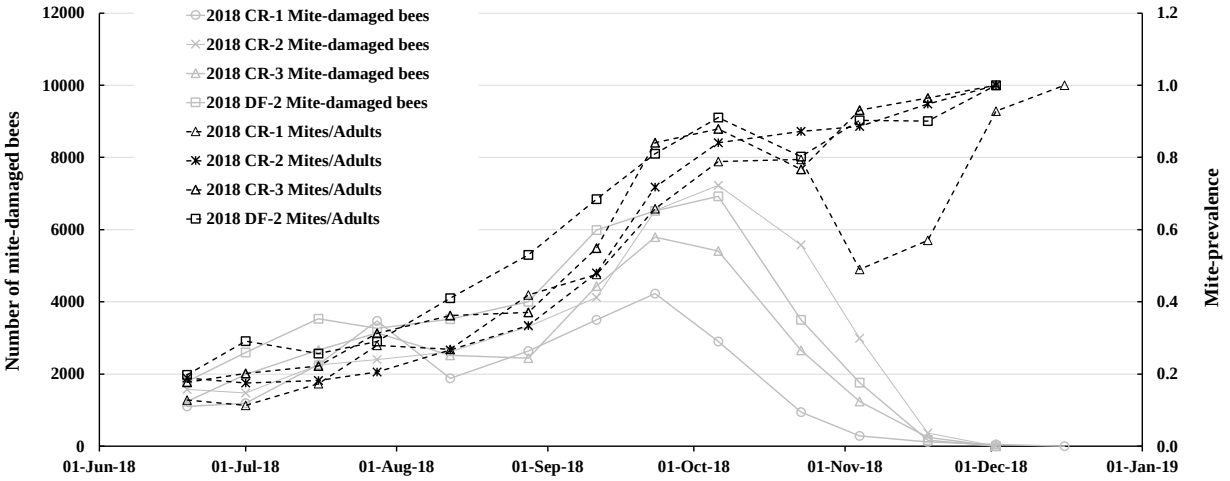

2018 CR-1 Mite-damaged bees
2018 CR-2 Mite-damaged bees
2018 CR-3 Mite-damaged bees
2018 DF-2 Mite-damaged bees
2018 CR-1 Mites/Adults
2018 CR-2 Mites/Adults
2018 CR-3 Mites/Adults
2018 DF-2 Mites/Adults
Number of mite-damaged bees
Mite-prevalence
12000
10000
8000
6000
4000
2000
0
1.2
1.0
0.8
0.6
0.4
0.2
0.0
01-Jun-18
01-Jul-18
01-Aug-18
01-Sep-18
01-Oct-18
01-Nov-18
01-Dec-18
01-Jan-19

Fig. S7. Apparent longevity of 2011/2012-Experiment.

CR-1 L(t): Apparent longevity of control colony (CR-1) to which pesticide is not administered. **DF 1ppm/syrup L(t):** Apparent longevity of experimental colony (DF-1) to which dinotefuran of 1 ppm is administered via sugar syrup. **DF 0.565ppm/pollen L(t):** Apparent longevity of experimental colony (DF-2) to which dinotefuran 0.565 ppm is administered via pollen paste. **DF 10ppm/syrup L(t):** Apparent longevity of experimental colony (DF-3) to which dinotefuran of 10 ppm is administered via sugar syrup. **DF 5.65ppm/pollen L(t):** Apparent longevity of experimental colony (DF-4) to which dinotefuran 5.65 ppm is administered via pollen paste. All colonies are mite-free.

The control colony, CR-1, and experimental colony, DF-3, begin to increase the apparent longevity from late September, as if they already have known that winter is approaching. CR-1 continues to increase during wintering, but DF-3 have become extinct soon after wintering. The extinction of DF-3 is thought to be due to the fact that the colony has been already weakened by dinotefuran before wintering, as evidenced by the fact that other experimental colonies, DF-1 and DF-4, became extinct before wintering.

On the other hand, as if another experimental colony, DF-2, does not notice that winter is approaching, its apparent longevity does not begin to increase from late September, and the longevity remains almost constant even into wintering. The almost constant longevity changes through the year for a bee colony are quite different from general seasonal longevity changes. This unusual change cannot be explained by the normal physiology of bees.

Although colonies fed sugar syrup containing dinotefuran become extinct earlier than pesticide-free colonies, pollen paste containing dinotefuran may have had some negative impact on the apparent longevity of bee colonies, judging by the fact that their apparent longevity is extended towards winter, just like pesticide-free colonies.

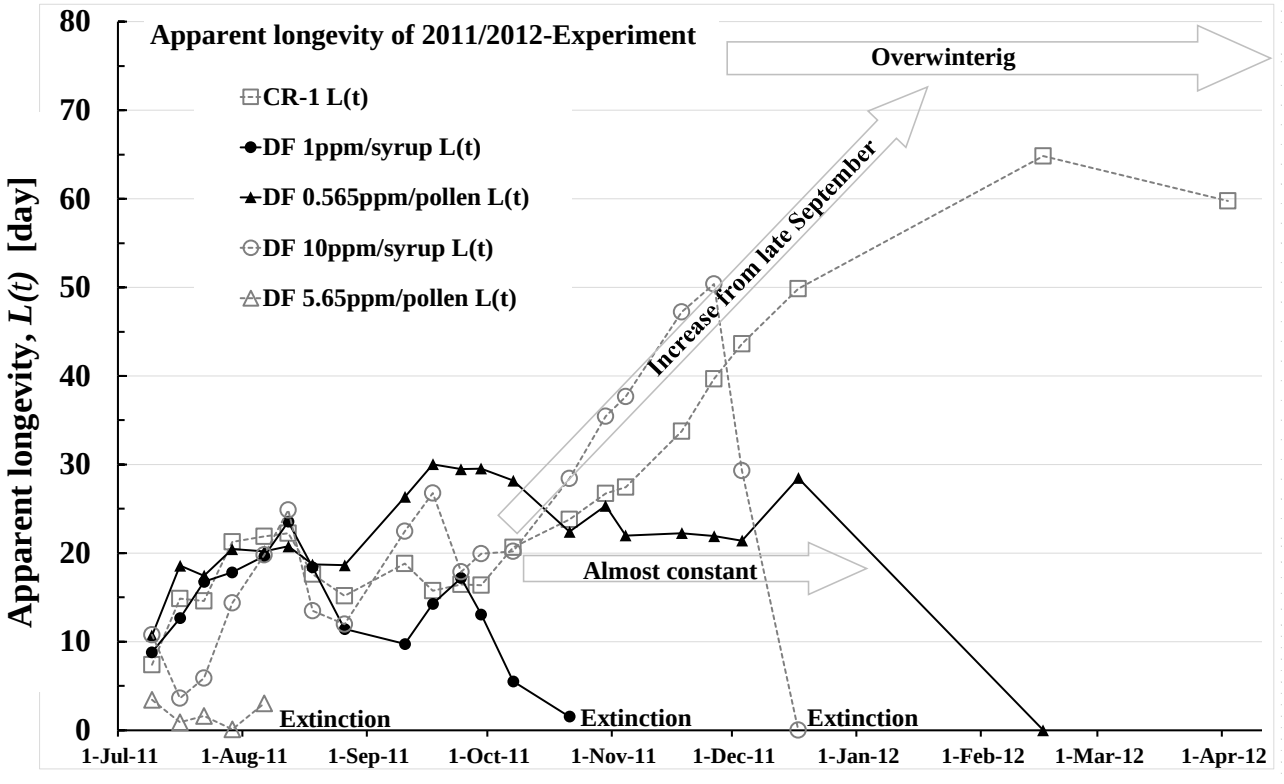

Apparent longevity of 2011/2012-Experiment
CR-1 L(t)
DF 1ppm/syrup L(t)
DF 0.565ppm/pollen L(t)
DF 10ppm/syrup L(t)
DF 5.65ppm/pollen L(t)
Overwinterig
Increase from late September
Almost constant
Extinction
Extinction
Extinction
Apparent longevity, L(t) [day]
80
70
60
50
40
30
20
10
0
1-Jul-11
1-Aug-11
1-Sep-11
1-Oct-11
1-Nov-11
1-Dec-11
1-Jan-12
1-Feb-12
1-Mar-12
1-Apr-12

Fig. S8. Apparent longevity of 2012/2013-Experiment.

CR-1 L(t) and CR-2 L(t): Apparent longevity of control colonies (CR-1, CR-2) to which pesticide is not administered. **DF 2ppm/syrup L(t):** Apparent longevity of experimental colony (DF-1) to which dinotefuran of 2 ppm is administered via sugar syrup. **FT10ppm/syrup L(t):** Apparent longevity of experimental colony (DF-2) to which fenitrothion of 10 ppm is administered via sugar syrup. All colonies are mite-free.

The experimental colony DF, became extinct early. Pesticide-free control colonies CR-1 and CR-2, as well as fenitrothion (organophosphate) containing colonies FT, exhibit general seasonal longevity changes. Its longevity is short and almost constant from the end of wintering to the end of September, then begins to extend from late September, continues to extend until just before the end of wintering, and when wintering is over, it suddenly drops to a short level, after which it stabilizes.

It is noteworthy that FT where is administered fenitrothion of an amount having same insecticidal power as 2 ppm dinotefuran succeeded in wintering, just like the control group. The difference between the early extinction of DF and the successful wintering of FT is probably due to the difference in the length of the residual effect period.

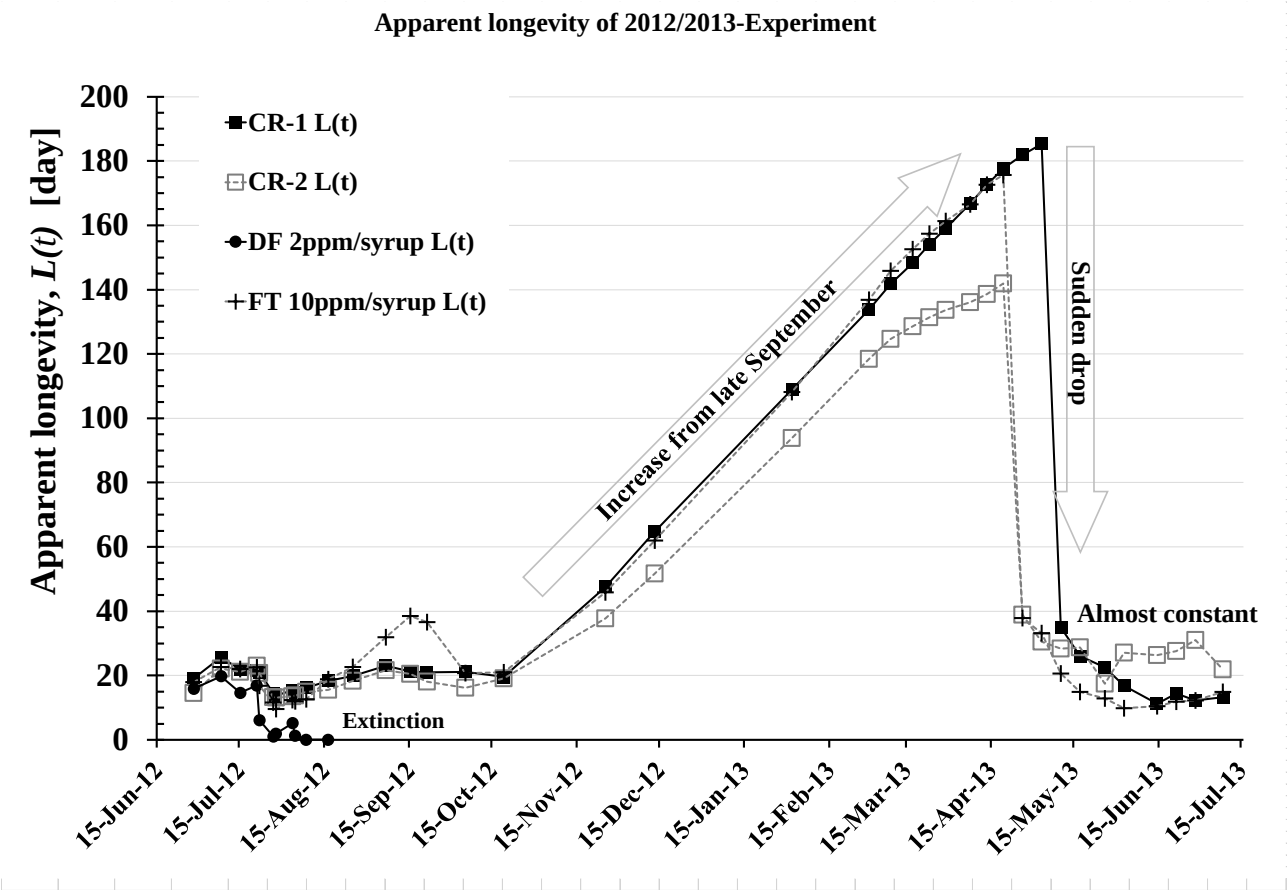

Apparent longevity of 2012/2013-Experiment
Apparent longevity, L(t) [day]
200
180
160
140
120
100
80
60
40
20
0
CR-1 L(t)
CR-2 L(t)
DF 2ppm/syrup L(t)
FT 10ppm/syrup L(t)
Increase from late September
Sudden drop
Almost constant
Extinction
15-Jun-12
15-Jul-12
15-Aug-12
15-Sep-12
15-Oct-12
15-Nov-12
15-Dec-12
15-Jan-13
15-Feb-13
15-Mar-13
15-Apr-13
15-May-13
15-Jun-13
15-Jul-13

Fig. S9. Apparent longevity of 2013/2014-Experiment.

CR-1(pesticide-free) L(t) and CR-2(pesticide-free) L(t): Apparent longevity of control colonies (CR-1, CR-2) to which pesticide is not administered. **DF 0.2 ppm/syrup L(t):** Apparent longevity of experimental colony (DF-1) to which dinotefuran of 0.2 ppm is administered via sugar syrup. **FT 1 ppm/syrup L(t):** Apparent longevity of experimental colony (DF-2) to which fenitrothion of 1 ppm is administered via sugar syrup. **MT 1 ppm/syrup L(t):** Apparent longevity of experimental colony (DF-2) to which malathion of 1 ppm is administered via sugar syrup. **CN 1 ppm/syrup L(t):** Apparent longevity of experimental colony (DF-2) to which clothianidin of 0.08 ppm is administered via sugar syrup. All colonies are mite-free.

The apparent longevity of all colonies (CR-1, CR-2, DF, FT, MT, CN) changed in the same way; it held almost constant at a short level from the start of experiment till late September, then began to extend toward winter, and continued to extend during wintering, but all colonies other than CR-2 became extinct during wintering.

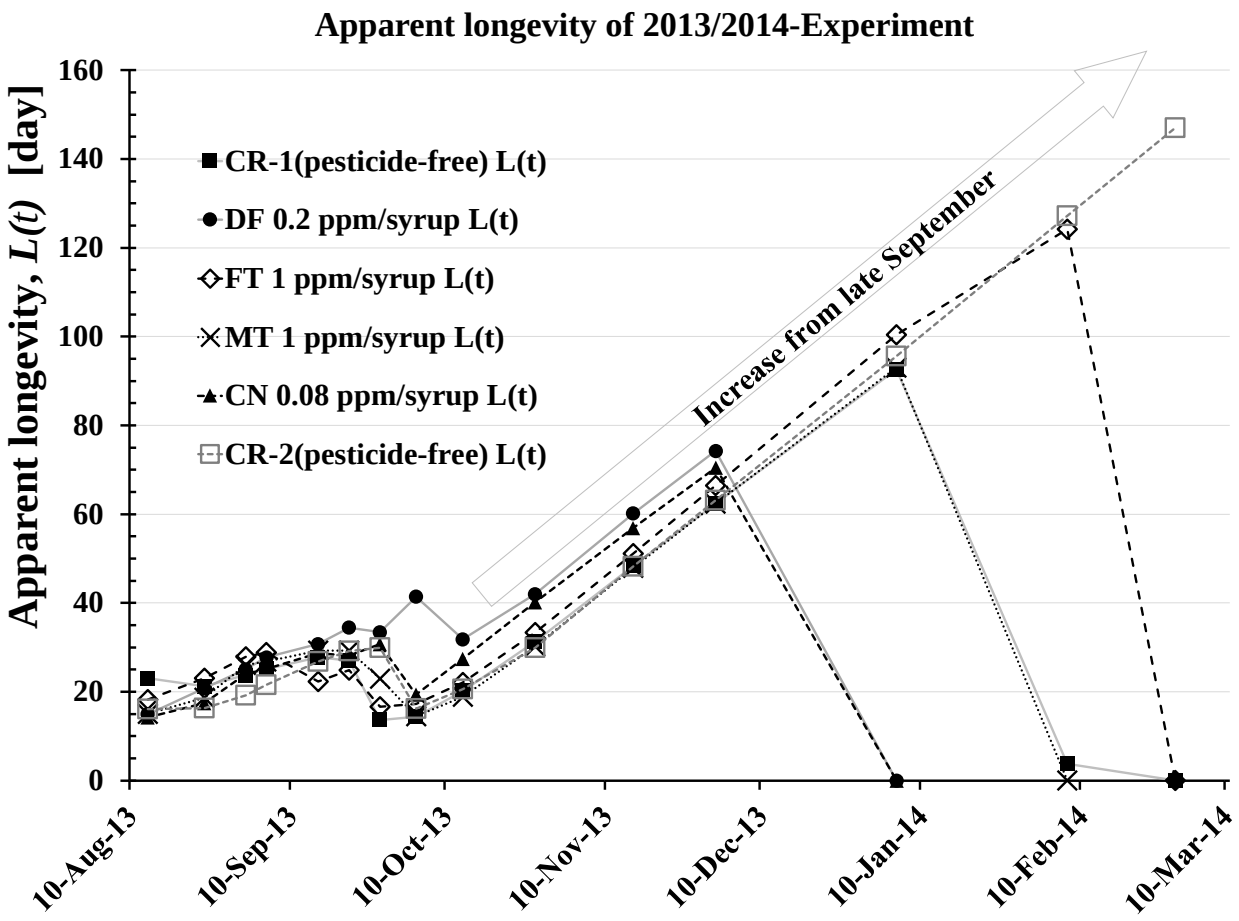
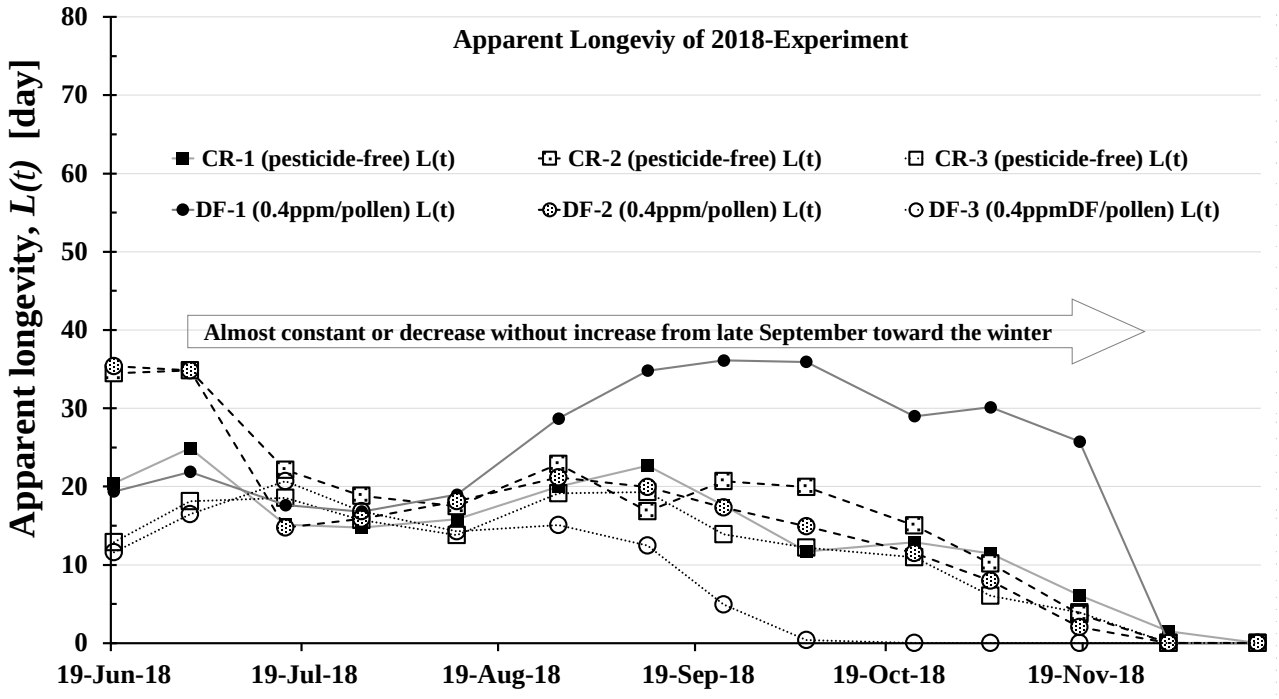

Apparent longevity of 2013/2014-Experiment
Apparent longevity, L(t) [day]
160
140
120
100
80
60
40
20
0
CR-1(pesticide-free) L(t)
DF 0.2 ppm/syrup L(t)
FT 1 ppm/syrup L(t)
MT 1 ppm/syrup L(t)
CN 0.08 ppm/syrup L(t)
CR-2(pesticide-free) L(t)
Increase from late September
10-Aug-13
10-Sep-13
10-Oct-13
10-Nov-13
10-Dec-13
10-Jan-14
10-Feb-14
10-Mar-14

Fig. S10. Apparent longevity of 2018-Experiment.

CR-1 (pesticide-free) L(t), CR-2 (pesticide-free) L(t) and CR-3 (pesticide-free) L(t): Apparent longevity of control colonies (CR-1, CR-2, CR-3) to which pesticide is not administered. **DF-1 (0.4 ppm/pollen) L(t), DF-2 (0.4 ppm/pollen) L(t) and DF-3 (0.4 ppm/pollen) L(t):** Apparent longevity of experimental colonies (DF-1, DF-2, DF-3) to which dinotefuran of 0.4 ppm is administered via pollen paste. All colonies were infested with mites (28).

All colonies do not have a characteristic change in apparent longevity that begins to extend from late September and continues to extend till the end of wintering, as commonly found in bee colonies. Interestingly, the apparent longevity of all colonies is, roughly speaking, fairly constant throughout the year, with little seasonal changes in apparent longevity. This is similar to the seasonal changes in the apparent longevity of bee colonies fed dinotefuran (neonicotinoid pesticides) via pollen paste (25).


Apparent Longeviy of 2018-Experiment
Apparent longevity, L(t) [day]
80
70
60
50
40
30
20
10
0
CR-1 (pesticide-free) L(t)
CR-2 (pesticide-free) L(t)
CR-3 (pesticide-free) L(t)
DF-1 (0.4ppm/pollen) L(t)
DF-2 (0.4ppm/pollen) L(t)
DF-3 (0.4ppmDF/pollen) L(t)
Almost constant or decrease without increase from late September toward the winter
19-Jun-18
19-Jul-18
19-Aug-18
19-Sep-18
19-Oct-18
19-Nov-18

3 Supplementary Tables (Tables.S1 to S7)

Table S1. Outline of pesticide intake in three field experiments.

Table S2. The essentials of experimental conditions and results for four long-term field experiments conducted in Shika, Japan.

Table S3. Apparent longevity of a honeybee colony in the 2011/2012 experiment at the eclosion rate of 0.9.

Table S4. Apparent longevity of a honeybee colony in the 2012/2013 experiment at the eclosion rate of 0.9.

Table S5. Apparent longevity of a honeybee colony in the 2013/2014 experiment at the eclosion rate of 0.9.

Table S6. Apparent longevity, $L(t)$, of a honeybee colony in the 2018 experiment at the eclosion rate of 0.9.

Table S7. Number of mite-damaged bees and mite-prevalence in 2018 and 2011/2012 experiments.

Table S1. Outline of pesticide intake in three field experiments.

Location of experimental site: mid-west Japan (Shika-machi) (Latitude 37°1'9"N; Longitude 136°46'14"N).

	Initial number of adult bees	Initial number of capped brood	Administration period of pesticide	Total intake of pesticide per colony during administration [mg/colony]	Total number of adult bees during administration	Total intake of pesticide per bee during administration [ng/bee]	Extinction or survival
2011/2012 CR-1 (pesticide-free)[25]				0		0	Survival
2011/2012 DF 1ppm/syrup[25]	2965	4263	9-Jul-11 to 21-Oct-11	4.208	13543	310.7	Extinction
2011/2012 DF-0.565ppm/pollen[25]	2158	2556	9-Jul-11 to 3-Dec-11	1.8692	30779	60.7	Extinction
2011/2012 DF 10ppm/syrup[25]	3296	3880	9-Jul-11 to 16-Jul-11	1.9	6344	290.3	Extinction
2011/2012 DF 5.65 ppm/pollen[25]	1659	6993	9-Jul-11 to 16-Jul-11	0.5257	3078	65.1	Extinction
2012/2013 CR-1 (pesticide-free)[26]				0		0	Survival
2012/2013 CR-2 (pesticide-free)[26]				0		0	Survival
2012/2013 DF 2ppm/syrup[26]	9173	9442	21-Jul-12 to 16-Aug-12	1.55	16524	93.8	Extinction
2012/2013 FT 10ppm/syrup[26]	8943	8732	21-Jul-12 to 16-Aug-12	17.97	19792	862.5	Survival
2013/2014 CR-1 (pesticide-free)[27]			0	0		0	Extinction
2013/2014 CR-2 (pesticide-free)[27]			0	0		0	Survival
2013/2014 DF 0.2ppm/syrup[27]	6017	2214	5-Sep-13 to 1-Dec-13	0.514	8612	56.7	Extinction
2013/2014 CN 0.08ppm/syrup[27]	7293	1506	5-Sep-13 to 1-Dec-13	0.4016	10130	39.6	Extinction
2013/2014 FT 1ppm/syrup[27]	10296	3232	5-Sep-13 to 28-Feb-14	3.86	19585	197.1	Extinction
2013/2014 MT 1ppm/syrup[27]	7676	1396	5-Sep-13 to 7-Feb-14	6.75	15724	429.3	Extinction

Table S2. The essentials of experimental conditions and results for four long-term field experiments conducted in Shika, Japan.

Experiment name **(Experimetal period)**	**2011/2012 experiment[25)]** **From July 9, 2011 to April 2, 2012 (268 days)**	**2012/2013 experiment[26)]** **From June 28, 2012 to July 26, 2013 (393 days)**	**2013/2014 experiment[27)]** **From Aug. 13, 2013 to Feb. 28, 2014 (199 days)**	**2018 experiment[28)]** **From Jul. 19, 2018 to Dec. 16, 2018 (180 days)**
Object of Study	To investigate the difference in the long-term impact on a bee colony between toxic sugar syrup (honey) as an energy source and toxic pollen pase (bee bread) as a protein source which are exposed to a neonicotinoid	To investigate the difference in the long-term impact on a bee colony between a neonicotinoid and a organophosphate which are administered through sugar syrup	To investigate the difference in the long-term impact on a bee colony between a neonicotinoid and a organophosphate which are administered through sugar syrup under lower concentrations than in the previous work	To investigate the seasonal changes in colony size and mite-prevalence in a honeybee colony exposed to dinotefuran via pollen paste and infested with *Varroa* mites
Circumstances around Experimental Site				
Experimental site (latitude and longitude)	Shika (mid-west Japan) (37°1'9" N, 136°46'14" E, 43m above sea level)	Same as the left	Same as the left	Shika (mid-west Japan) (37 °2 '35" N, 136 °45 '38" E, 70m above sea level)
Limitation of honeybee activities	Honeybees can freely forage about for food in a hive or in fields	Same as the left	Same as the left	Same as the left
Environmental effects of pesticides other than administered pesticides	A pesticide-free watering place & a pesticide-free field of flowers in the apiary	Same as the left	Same as the left	Same as the left
Aerial-crop-dusting farmland near experimental site	Nothing	Nothing	Nothing	Nothing
Seasonal changes	Distinct	Distinct	Distinct	Distinct
Experimental Conditions				
Materials	Vehicle : sugar syrup; pollen paste. Pesticide: dinotefuran	Vehicle: sugar syrup; pollen paste. Pesticide: dinotefuran;fenitrothion	Vehicle: sugar syrup; pollen paste. Pesticide: dinotefuran; clothianidin; fenitrothion; malathion	Vehicle: sugar syrup; pollen paste. Pesticide: dinotefuran
Initial numbers of apiaries, colonies and combs (frames)	One apiary (private), five colonies per apiary, three combs per colony (one colony for each same conditions)	One apiary (private), four colonies per apiary, three combs per colony (one colony for each same conditions except 2 controls)	One apiary (private), six colonies per apiary, three combs per colony (one colony for each same conditions except 2 controls)	One apiary (private), six colonies per apiary, three combs per colony (3 control colonies & 3 dinotefuran-exposed colonies)
Concentration of pesticide	Dinotefuran: 1 & 10 ppm in sugar syrup, 0.565 & 5.65 in pollen paste	Dinotefuran: 2 ppm in sugar syrup. Fenitrothion: 10 ppm in sugar syrup	Dinotefuran: 0.2 ppm in sugar syrup. Clothianidin: 0.08 ppm in sugar syrup. Fenitrothion & Malathion: 1 ppm in sugar syrup	Dinotefuran: 0.4 ppm in pollen paste
Initial number of bees per colony	1700 to 3400 bees (accurately counted from photos)	5600 to 7100 bees (accurately counted on photos)	5400 to 7600 bees (accurately counted on photos)	7000 to 9000 bees (accurately counted on photos)
Initial number of capped brood per colony	2600 to 6100 capped brood (accurately counted from photos)	4000 to 5700 capped brood (accurately counted from photos)	4200 to 7600 capped brood (accurately counted from photos)	3100 to 6900 capped brood (accurately counted from photos)
Origin of a queen	Unknown in detail (Apis mellifera) Bee colonies purchased from a bee farm	Same as the left	Same as the left	Queen bees in sister relationship
Experimental Methods				
Interval of experiment	About one-week interval	About one-week or two-weeks interval	Same as left	About two-weeks inter
Administration period of pesticide	From July 9, 2011 to the colony extinction, or to December 3 (147 days)	From July 21, 2012 to the colony extinction, or to August 16 (26 days)	From September 5, 2013 to the colony extinction, or to December 1 (87 days)	From July 1, 2018 to the colony extinction
Starting time of each observation	Just after dawn if possible (before bees go out to forage)	Same as the left	Same as the left	Same as the left
Vehicle to administer a pesticide	Either sugar Syrup or pollen paste	Sugar syrup	Sugar syrup	pollen paste
Administration method of pesticide	A pesticide was dissolved in sugar syrup or pollen was kneaded with toxic sugar syrup containing the pesticide. Either toxic sugar syrup or toxic pollen paste was fed into a hive	A pesticide was dissolved in sugar syrup with toxic sugar syrup containing the pesticide. Only toxic sugar syrup was fed into a hive.	A pesticide was dissolved in sugar syrup with toxic sugar syrup containing the pesticide. Only toxic sugar syrup was continuously fed into a hive with an auto-feeding system composed of 10 L (14 kg syrup) container	Pollen paste was made while pollen was being kneaded with toxic sugar syrup containing the pesticide. Toxic pollen paste was fed into a hive.
Counting method of the number of adult bees	Directly counted with accuracy from photos of combs with bees and bees left in a hive after every comb was removed from it with the help of a automatic counting software	Same as the left (auxiliary automatic counting software has been improved)	Same as the left (auxiliary automatic counting software has been improved)	Same as the left (auxiliary automatic counting software has been improved)
Counting method of the number of capped brood	Acurately counted from photos of combs without bees after shaking the bees off each comb with the help of a automatic counting software	Same as the left (auxiliary automatic counting software has been improved)	Same as the left (auxiliary automatic counting software has been improved)	Same as the left (auxiliary automatic counting software has been improved)
Total intake of pesticide per colony	Calculated from the sugar syrup or pollen paste with pesticide consumed by honeybees.	Calculated from the sugar syrup with pesticide consumed by honeybees	Same as the left	Same as the left
Estimation of the intake of pesticide per bee	Estimated from dividing the total intake of pesticide per colony by the total number of initial & newly-emerged honeybees	Same as the left	Same as the left	Same as the left
Counting method of number of dead bees	Dead bees were accurately counted one by one inside and outside a hive which was placed on a large tray	Same as the left	Same as the left	Same as the left
Confirmation and record methods of a queen	A photographic record of the exsistence of a queen in each colony	Same as the left	Same as the left	Same as the left
Publications of Research Results (Title, Journal, Short Abstract)				
Experimental Results	A clear difference in the impact on honeybee (*Apis mellifera*) colony between the two vehicles of sugar syrup and pollen paste	Difference between the impact of the neonicotinoid dinotefuran and organophosphate fenitrothion on a bee colony in a long-term field experiment: An evidence	Comparison of the influence of a pesticide at an environmentally realistic concentration level in Japan on a honeybee colony between neonicotinoids (dinotefuran, clothianidin) and organophosphates (fenitrothion, malathion)	Seasonal changes in the size and mite-prevalence of a bee colony exposed to dinotefuran via pollen paste and damaged by Varroa mites
	T. Yamada et al. (2018). *J. Biol. Ser.* 1(3): 084-107.	Yamada et al. (2018). *J. Biol. Ser.* 1(3): 108-137	T. Yamada et al. (2018). *J. Biol. Ser.* 1(4): 187-207.	Yamada T. (2020). *J. Diet. Res. Nutr.* 7(1): 002.
	This study investigates the difference in the impact of the neonicotinoid pesticide dinotefuran on honeybee colony between the two vehicles of sugar syrup and pollen paste. A distinct difference was observed between the two vehicles: The per-bee intake of dinotefuran administered through pollen paste as a vehicle until colony extinction was roughly one-fifth of the per-bee intake administered through sugar syrup, independently of dinotefuran concentrations. This difference can be attributed to the dissimilarity in strength of the impact on honeybee colony between worker bees which preferentially take sugar syrup (honey) to pollen paste and a queen bee and brood (larvae) which take pollen paste (bee bread) in preference to sugar syrup as a result of the long-lasting toxicity of dinotefuran. This suggests that pollen as a protein source contaminated by neonicotinoid pesticides can cause deeper adverse effect on a honeybee colony than honey as an energy source.	We conducted a long-term field experiment and found different impacts on honeybee colonies in an apiary between the neonicotinoid dinotefuran and the organophosphate fenitrothion. The colony where dinotefran was administered (dinotefuran colony) became extinct in the administration period of 26 days, while the colony where fenitrothion was administered (fenitrothion colony) survived long after the same administration period. The fenitrothion colony succeeded in overwintering and staying alive for more than 293 days after administration. We speculate that colonies exposed to dinotefuran hardly recover from the damage because dinotefuran has a much longer persistent ability than fenitrothion and toxic foods stored in cells over a prolonged period of time can affect a colony.	In this paper the differences are investigated between neonicotinoids (dinotefuran, clothianidin) and organophosphates (fenitrothion, malathion) on a realistic pesticide-concentration level in the natural environment surrounding an apiary in Japan. It has been shown that neonicotinoids can much more rapidly weaken the colony where it was administered than organophosphates and organophosphates can be rapidly degraded in honey stored because of their high degradability. These results roughly reproduced the findings in our previous experiment. Neonicotinoid-concentration in residual honey in comb-cells were lower than those in sugar syrup fed to each colony were detected but organophosphates were hardly detected. We inferred that obscure massive colony losses in winter can be probably caused by toxic food with a long-term persistent pesticide such as a neonicotinoid stored in combs during overwintering after the weakening of the colony due to the ingestion of toxic nectar, pollen and water under the natural circumstances contaminated by long-persistent pesticides such as neonicotinoids just before	In this study, the impact of dinotefuran (DF) administered via pollen paste to a bee colony damaged by Varroa mites was investigated. Seasonal changes in the number of adult bees (those capped brood) and the mite-prevalence among adult bees were measured through a field experiment over 180 days. It was found that the bee colony collapsed under the intake of a smaller amount of DF due to the synergistic effect of DF and mite-damage. Because the daily pesticide-free sugar-syrup intake per bee in the DF-exposed colony administered via pollen paste was greater than that of the control colony, DF may have an appetite-promoting effect. Since the consumption of DF-containing pollen paste per bee per day showed almost no difference between all colonies, DF seems to have no repellent effect. Mite-prevalence continued to rise and became almost 100% near the time of colony extinction. The inner-temperature-fluctuation-range of the hive-box (Ti) was smaller than that of the ambient temperature (Ta). The inner-temperature-fluctuation-range of the DF-exposed-colony hive-box was larger
Estimated results of apparent longevity	A mathematical model to estimate the seasonal change in apparent longevity of bee colony			
	Y. Yamada et al. (2019). *Scientific Reports*, volume 9, Article number: 4102 (2019)			
	We have proposed a new mathematical model to estimate the apparent longevity defined in the upper limit of an integral equation. The apparent longevity can be determined only from the numbers of adult bees and capped brood. By applying the mathematical model to a honeybee colony in Japan, seasonal changes in apparent longevity were estimated in three long-term field experiments. Three apparent longevities showed very similar season-changes to one another, increasing from early autumn, reaching a maximum at the end of overwintering and falling approximately plumb down after overwintering. (This study was performed using only the control colonies.)			
	Damage to larvae causes seasonal changes in honeybee longevity to go crazy			
	T.Yamada and Y. Yamada., preparing paper submission (this work)			
	The followings were clarified from the honeybee longevity estimated by the mathematical model using the numbers of adult bees and capped brood obtained from three long-term field experiments. The longevities of the mite-free control colony and the mite-free colony where a pesticide is administered via sugar syrup show common seasonal changes, which continue to increase from the end of September to the end of overwintering and drop rapidly just after overwintering, though the ingestion of a pesticide via sugar syrup shortens the honeybee longevity. However, the longevity of the mite-infested colony and that of the mite-free colony where a pesticide is administered via pollen paste show abnormal seasonal changes in longevity that cannot increase since late September. Such abnormal seasonal changes may be caused by biological rhythm disorders due to damages by toxic pollen paste and parasitic mites during the brood stage when seasonal sensory functions and organs are created. We infer that such damages to larvae and pupae can disrupt bees' internal clocks and make honeybees undetectable of the arrival of winter, and make the colony fail in overwintering because the longevity does not extend due to abnormal seasonal changes in longevity. (This study was performed using all the colonies other than those that collapsed before mid-November)			

Table S3. Apparent longevity of a honeybee colony in the 2011/2012 experiment at the eclosion rate of 0.9.

This table shows the data source of the apparent longevity in Fig.1, which were estimated using the field experimental results conducted in 2011 to 2012. *a(t)* and *b(t)* denote the number of adult bees and the number of capped brood obtained from the field experiment conducted from 2011 to 2012, respectively (*25*). *L(t)* is the apparent longevity estimated using *a(t)* and *b(t)* by the mathematical model previously proposed (*1*). "CR-1" denotes the control colony where mites are almost absent and a pesticide is not administered. "DF 1ppm/syrup" and "DF 10ppm/syrup" denote the colonies where the neonicotinoid dinotefuran of 1 ppm and 10 ppm are administered via sugar syrup, respectively. "DF 0.565ppm/pollen" and "DF 5.65ppm/pollen" denote the colonies where the neonicotinoid dinotefuran of 0.565 ppm and 5.65 are administered via pollen paste, respectively. **Note:** A pesticide-administration period is from the morning of July 9 in 2011 until a colony becomes extinct, but until the morning of December 3 when a colony survives to the winter (*25*).

Date	Elapsed days	CR-1			DF 1ppm/syrup			DF 0.565ppm/pollen			DF 10ppm/syrup			DF 5.65ppm/pollen		
		a(t)	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*
9-Jul-11	0	3392	5819	7.38	2965	4263	8.80	2158	2556	10.68	3295	3880	10.75	1659	6093	3.45
16-Jul-11	7	6827	3644	14.85	4257	1287	12.64	3755	2377	18.59	1102	986	3.59	430	2865	0.89
22-Jul-11	13	6399	4692	14.59	5233	1655	16.75	3493	3207	17.45	1407	232	5.88	330	1165	1.62
29-Jul-11	20	7737	4861	21.27	3131	1034	17.82	4025	2743	20.45	1451	939	14.38	17	790	0.12
6-Aug-11	28	7893	7179	21.89	2393	1591	19.66	4372	3480	20.21	1384	2370	19.78	1	729	3.02
12-Aug-11	34	7675	8390	22.26	1762	2288	23.55	4513	3217	20.77	1752	3217	24.85			
18-Aug-11	40	8873	6125	17.63	2298	1137	18.38	4829	1933	18.72	2257	946	13.50			
26-Aug-11	48	9327	5797	15.17	1623	1757	11.42	4276	2099	18.63	2271	913	11.99			
10-Sep-11	63	9249	8803	18.82	1253	318	9.73	4620	1343	26.35	2096	545	22.46			
17-Sep-11	70	9762	8327	15.75	997	341	14.26	4241	1546	30.04	1760	707	26.76			
24-Sep-11	77	11252	7034	16.44	462	453	17.18	3862	2693	29.47	908	772	17.90			
29-Sep-11	82	10736	5810	16.37	342	230	13.06	3792	2445	29.54	996	505	19.94			
7-Oct-11	90	12015	6100	20.65	169	109	5.51	4232	2082	28.18	1076	12	20.19			
21-Oct-11	104	11253	4100	23.79	0	45	1.53	4225	1935	22.40	860	220	28.40			
30-Oct-11	113	10958	4300	26.73				4044	2105	25.35	895	147	35.46			
4-Nov-11	118	10654	3900	27.44				3675	2665	21.99	790	245	37.66			
18-Nov-11	132	11303	3430	33.75				4022	1720	22.25	779	160	47.23			
26-Nov-11	140	12390	1750	39.66				3806	1034	21.91	630	48	50.39			
3-Dec-11	148	12109	2038	43.64				2992	840	21.41	393	21	29.30			
17-Dec-11	162	11811	2038	49.87				2433	157	28.47	0	0	0.00			
16-Feb-12	223	10514	2038	64.81				0	0	0.00						
2-Apr-12	269	9622	2038	59.75												

Table S4. Apparent longevity of a honeybee colony in the 2012/2013 experiment at the eclosion rate of 0.9.

This table shows the data source of the apparent longevity in Fig.1, which were estimated using the field experimental results conducted in 2012 to 2013. *a(t)* and *b(t)* denote the number of adult bees and the number of capped brood obtained from the field experiment conducted from 2012 to 2013, respectively (*26*). *L(t)* is the apparent longevity estimated using *a(t)* and *b(t)* by the mathematical model previously proposed (*1*). "CR-1" and "CR-2" denote the control colonies where mites are almost absent and a pesticide is not administered, respectively. "DF 2ppm/syrup" denotes the colonies where the neonicotinoid dinotefuran of 2 ppm is administered via sugar syrup and mites are almost absent. "FT 10ppm/syrup" denotes the colony where the organophosphate fenitrothion of 10 ppm is administered via sugar syrup and mites are almost absent. **Note:** A pesticide-administration period is from the morning of July 21 in 2012 until the morning of August 16 (*26*).

Date	Elapsed days	CR-1			CR-2			DF 2ppm/syrup			FT 10ppm/syrup		
		a(t)	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*
28-Jun-12	0	7136	4746	19.03	5832	5094	14.49	7119	5679	15.86	5690	4012	17.95
8-Jul-12	10	9621	5806	25.65	8917	4710	22.15	8877	6446	19.78	7157	5286	22.58
15-Jul-12	17	8695	10215	21.94	8265	11143	21.07	6878	13267	14.61	7565	9619	22.06
21-Jul-12	23	9647	10254	21.53	9665	11301	23.06	9173	9442	16.91	8943	8732	22.56
22-Jul-12	24	10136	10210	21.12	9558	10967	20.76	4885	8834	6.11	7750	8694	16.76
27-Jul-12	29	10633	10617	14.51	10770	10329	13.24	1434	4548	1.03	8721	6563	11.57
28-Jul-12	30	10391	10858	13.79	10901	10581	13.15	1313	3891	1.90	7786	6389	9.52
3-Aug-12	36	12083	10000	15.41	11939	10025	13.52	1049	1131	5.15	8289	3390	12.31
4-Aug-12	37	12389	9687	14.88	12041	10269	13.70	771	840	1.22	7559	2901	11.96
8-Aug-12	41	14065	7154	16.23	12978	8472	14.86	33	208	0.05	6625	1352	12.62
16-Aug-12	49	13371	6111	18.39	12207	5977	15.60	0	0	0.00	5961	607	18.86
25-Aug-12	58	11961	6014	19.88	10997	6684	18.37				4467	918	22.64
6-Sep-12	70	11165	8783	23.00	11582	8126	21.68				3534	3406	31.86
15-Sep-12	79	11980	5531	21.31	11825	7135	20.47				4576	4187	38.45
21-Sep-12	85	12166	6086	21.00	11025	9202	18.04				5859	4119	36.60
5-Oct-12	99	10715	7615	21.14	10510	5679	16.13				6593	4648	20.80
19-Oct-12	113	11726	7280	19.59	10038	6628	19.03				7326	4713	20.95
25-Nov-12	150	13255	36	47.53	12477	2937	37.74				7755	10	45.86
13-Dec-12	168	12858	0	64.81	13316	305	51.75				7080	0	62.00
1-Feb-13	218	9306	0	108.91	9421	0	93.76				5652	0	108.11
1-Mar-13	246	7464	17	133.75	8426	0	118.44				5957	26	136.85
9-Mar-13	254	7512	497	141.82	8017	660	124.67				6358	619	145.90
17-Mar-13	262	6862	1691	148.28	7372	2419	128.57				6152	2329	152.54
23-Mar-13	268	7312	2431	154.08	7416	3624	131.34				6470	3217	157.36
29-Mar-13	274	7720	4097	159.04	8018	5165	133.66				7143	4994	161.37
7-Apr-13	283	9518	7326	166.80	9833	8414	136.13				8797	7053	166.52
13-Apr-13	289	12523	10166	172.59	12594	11211	138.63				12038	8670	172.62
19-Apr-13	295	15677	11324	177.64	16221	12975	141.88				14275	10320	175.61
26-Apr-13	302	20574	9725	181.88	20412	14287	38.93				17132	10803	37.89
3-May-13	309	23935	5808	185.32	24100	14521	30.47				20413	9631	33.22
10-May-13	316	23629	4551	34.93	27670	13380	28.37				16477	9020	20.60
17-May-13	323	16210	6036	26.01	30325	12864	28.76				11103	8732	14.91
26-May-13	332	9981	9778	22.57	17904	10157	17.50				8817	6867	12.86
2-Jun-13	339	9289	7396	16.76	26814	3454	27.14				5763	5069	9.89
14-Jun-13	351	6135	4875	11.18	16284	1600	26.34				3936	2720	10.49
21-Jun-13	358	5590	3089	14.36	11501	7725	27.64				3058	1706	11.92
28-Jun-13	365	4210	1522	12.24	10454	10165	31.03				2336	1477	12.21
8-Jul-13	375	1962	978	13.31	11162	7534	21.93				1839	796	14.87
26-Jul-13	393				4817	4498	10.72						

Table S5. Apparent longevity of a honeybee colony in the 2013/2014 experiment at the eclosion rate of 0.9.

This table shows the data source of the apparent longevity in Fig.1, which were estimated using the field experimental results conducted in 2013 to 2014. *a(t)* and *b(t)* denote the number of adult bees and the number of capped brood obtained from the field experiment conducted from 2012 to 2013, respectively (*27*). *L(t)* is the apparent longevity estimated using *a(t)* and *b(t)* by the mathematical model previously proposed (*1*). "CR-1" and "CR-2" denotes the mite-free control colonies where a pesticide is not administered. "DF 0.2ppm/syrup" denotes the mite-free colony where the neonicotinoid dinotefuran of 0.2 ppm is administered via sugar syrup. "CN 0.0.08ppm/syrup" denotes the mite-free colony where the neonicotinoid clothianidin of 0.08 ppm is administered via sugar syrup. "FT 1ppm/syrup" denotes the mite-free colony where the organophosphate fenitrothion of 1 ppm is administered via sugar syrup. "MT 1ppm/syrup" denotes the mite-free colony where the organophosphate malathion of 1 ppm is administered via sugar syrup. **Note :** A pesticide-administration period is from the morning of September 5 in 2013 until December 1 (*27*).

Date	Elapsed days	CR-1			DF 0.2 ppm/syrup			FT 1 ppm/syrup			MT 1 ppm/syrup			CN 0.08 ppm/syrup			CR-2		
		a(t)	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*
13-Aug-13	0	7579	4167	23.01	5447	4605	14.97	7360	5094	18.28	5500	4616	15.08	5628	5022	14.18	5953	4683	16.09
24-Aug-13	11	7000	3363	21.26	7556	323	20.76	9290	3758	23.08	6803	2414	18.65	6915	2208	17.43	6041	2146	16.32
1-Sep-13	19	7324	1825	23.77	6529	1219	25.07	10402	2671	27.90	8177	1054	26.11	7790	1025	23.95	5565	558	19.22
5-Sep-13	23	7487	2261	25.14	6017	2214	27.74	10296	3232	28.81	7676	1396	26.84	7293	1506	25.17	5549	578	21.56
15-Sep-13	33	6415	5462	27.73	5072	1743	30.73	5795	6040	22.27	5716	4992	29.32	5574	1825	28.67	4027	2665	26.86
21-Sep-13	39	6697	4683	27.08	5154	90	34.44	7431	3015	24.86	5698	3943	29.32	4001	843	28.20	3829	3198	29.13
27-Sep-13	45	5711	2538	13.68	3317	0	33.45	6920	1655	16.68	5617	2456	22.93	3885	208	30.74	3889	2882	29.88
4-Oct-13	52	5048	649	14.39	3145	0	41.41	5124	1456	17.33	4445	984	14.47	1882	160	19.35	3824	2142	16.20
13-Oct-13	61	4210	151	20.18	2029	0	31.82	4478	11	22.14	3459	0	18.90	1762	7	27.41	4322	113	20.63
27-Oct-13	75	3152	314	31.34	1325	0	41.98	3147	0	33.36	2328	0	30.19	1449	0	40.19	3371	0	29.89
15-Nov-13	94	2714	50	48.37	1146	0	60.17	2554	0	51.13	2050	0	47.95	1143	0	56.81	3181	0	48.19
1-Dec-13	110	2189	3	62.34	795	0	74.23	2213	0	66.43	1745	0	62.35	867	0	70.46	2904	0	63.18
5-Jan-14	145	1100	0	92.61	0	0	0.00	1714	0	100.39	916	0	92.97	0	0	0.00	2218	0	95.60
7-Feb-14	178	0	314	3.81				878	0	124.23	0	0	0.00				1799	0	127.15
28-Feb-14	199	0		0.00				0	0	0.00							1594	0	147.03

Table S6. Apparent longevity, *L(t)*, of a honeybee colony in the 2018 experiment at the eclosion rate of 0.9.

This table shows the data source of the apparent longevity in Fig.1, which were estimated using the field experimental results conducted in 2018. *a(t)* and *b(t)* denote the number of adult bees and the number of capped brood obtained from the field experiment conducted from 2012 to 2013, respectively (*28*). *L(t)* is the apparent longevity estimated using *a(t)* and *b(t)* by the mathematical model previously proposed (*1*). "CR-1", "CR-2" and "CR-3" denote the mite-infested control colonies where a pesticide is not administered. "DF-1 0.4ppm/syrup", "DF-2 0.4ppm/syrup" and "DF-3 0.4ppm/syrup" denote the mite-infested colonies where the neonicotinoid dinotefuran of 0.4 ppm is administered via pollen paste. Note: A pesticide-administration period is from the morning of July 1 in 2018 until the colony extinction (*28*).

Date	Elapsed days	CR-1			CR-2			CR-3			DF-1 0.4ppm/pollen			DF-2 0.4ppm/pollen			DF-3 0.4ppm/pollen		
		a(t)	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*	*a(t)*	*b(t)*	*L(t)*
19-Jun-18	0	8613	5341	20.41	8343	3065	34.45	6987	6861	12.89	9010	5891	19.36	9067	3241	35.40	7079	7726	678.30
1-Jul-18	12	10507	11056	24.90	8439	9401	34.84	9830	8621	18.13	10188	9341	21.89	8926	12549	34.85	10071	5374	471.80
16-Jul-18	27	13064	10657	15.13	12402	7447	22.11	12016	8060	18.56	12070	8360	17.64	13726	8755	14.75	10491	8032	705.20
28-Jul-18	39	12439	5688	14.77	11658	7640	18.81	10037	7006	15.71	11239	5443	16.80	11252	5769	15.88	10301	9052	794.80
12-Aug-18	54	6977	3817	15.81	9812	4619	17.50	6965	4078	13.83	8437	2777	18.96	8588	4030	18.13	9213	4840	425.00
28-Aug-18	70	6284	4125	20.01	9902	6629	22.86	6583	5277	19.15	7609	466	28.70	7558	5690	21.16	5662	4504	395.50
11-Sep-18	84	7369	4755	22.65	8589	5050	16.87	8081	6262	19.30	4716	395	34.80	8742	5928	19.92	4204	2978	261.50
23-Sep-18	96	6427	4201	17.58	9102	5636	20.71	6891	6487	13.93	2370	200	36.12	8046	6559	17.31	1158	1266	111.20
6-Oct-18	109	3680	1490	11.73	8593	5982	19.96	6150	5015	12.22	964	105	35.94	7599	6091	14.92	41	1228	107.80
23-Oct-18	126	1187	658	12.87	6395	4200	15.02	3461	2773	10.97	309	70	28.98	4366	3113	11.52	0	1234	108.30
4-Nov-18	138	596	440	11.46	3373	1747	10.16	1332	1043	6.08	204	43	30.13	1957	1646	7.96	0	0	0.10
18-Nov-18	152	207	411	6.09	383	913	3.73	254	607	3.92	112	39	25.76	191	1147	2.07	0	0	0.00
2-Dec-18	166	56	460	1.54	0	989	0.00	0	691	0.00	0	72	0.00	0	1291	0.00	0	0	0.00
16-Dec-18	180	0	463	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00

Table S7. Number of mite-damaged bees and mite-prevalence in 2018 and 2011/2012 experiments

2018 (*28*) and 2011/2012 (*25*) denote the years when the field experiments were conducted, respectively. CR-1, CR-2 and CR-3 denote the control colonies where no pesticide was administered in the 2018 experiment (*28*), respectively. DF-1, DF-2 and DF-3 denote the colonies where dinotefuran of 0.4 ppm was administered through pollen paste in the 2018 experiment (*28*), respectively. DF denotes the colony where dinotefuran of 0.565 ppm was administered through pollen paste in 2011/2012 experiment (*25*). Mites and Adults denote the number of mite-damaged bees and the number of adult bees, respectively. Mite-prev. denotes the mite-prevalence which is given by dividing the number of adult bees (Adults) into the number of mite-damaged bees (Mites). Incidentally, the numbers of mite-damaged bees (Mites) and adult bees (Adults) and the mite-prevalence (Mite-prev.) in the 2018 experiment (*28*) and the number of adult bees (Adults) in the 2011/2012 experiment (*25*) have already been reported. The number of mite-damaged bees (Mites) and the mite-prevalence (Mite-prev.) in the 2011/2012 experiment were newly measured in this work using the photograph images (*25*) taken in the observation dates selected at intervals of about one month.

	2018																		2011/2012		
Date of observation	CR-1			CR-2			CR-3			DF-1			DF-2			DF-3			DF		
	Mites	Adults	Mite-prev.	Mites	Adults	Mite-prev.	Mites	Adults	Mite-prev.	Mites	Adults	Mite-prev.	Mites	Adults	Mite-prev.	Mites	Adults	Mite-prev.	Mites	Adults	Mite-prev.
19-Jun	1103	8613	0.1281	1577	8343	0.1890	1237	6987	0.1770	1281	9010	0.1422	1795	9067	0.1980	1940	7079	0.2741			
1-Jul	1190	10507	0.1133	1482	8439	0.1756	1986	9830	0.2020	1739	10188	0.1707	2603	8926	0.2916	2457	10071	0.2440			
9-Jul																			84	2158	0.0389
16-Jul	2263	13064	0.1732	2255	12402	0.1818	2670	12016	0.2222	2380	12070	0.1972	3532	13726	0.2573	3488	10491	0.3325			
28-Jul	3475	12439	0.2794	2401	11658	0.2060	3142	10037	0.3130	3351	11239	0.2982	3269	11252	0.2905	3881	10301	0.3768			
6-Aug																			112	4372	0.0256
12-Aug	1876	6977	0.2689	2620	9812	0.2670	2523	6965	0.3622	2689	8437	0.3187	3519	8588	0.4098	3819	9213	0.4145			
28-Aug	2636	6284	0.4195	3313	9902	0.3346	2442	6583	0.3710	2643	7609	0.3474	4008	7558	0.5303	2378	5662	0.4200			
10-Sep																			90	4620	0.0195
11-Sep	3507	7369	0.4759	4125	8589	0.4803	4438	8081	0.5492	3229	4716	0.6847	5988	8742	0.6850	3072	4204	0.7307			
23-Sep	4230	6427	0.6582	6531	9102	0.7175	5793	6891	0.8407	1985	2370	0.8376	6523	8046	0.8107	1002	1158	0.8653			
6-Oct	2903	3680	0.7889	7227	8593	0.8410	5408	6150	0.8793	815	964	0.8454	6919	7599	0.9105	39	41	0.9512			
7-Oct																			104	4232	0.0246
23-Oct	943	1187	0.7944	5575	6395	0.8718	2654	3461	0.7668	247	309	0.7994	3502	4366	0.8021	1	0	1.0000			
4-Nov	292	596	0.4899	2989	3373	0.8862	1240	1332	0.9309	198	204	0.9706	1766	1957	0.9024	0	0		111	3675	0.0302
18-Nov	118	207	0.5700	363	383	0.9478	245	254	0.9646	100	112	0.8929	172	191	0.9005	0	0				
2-Dec	52	56	0.9286	1	0	1.0000	1	0	1.0000	3	0	1.0000	1	0	1.0000	0	0				
3-Dec																			144	2992	0.0481
16-Dec	0	0	1.0000													0	0				

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