



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

A gene drive does not spread easily in populations of the honey bee parasite *Varroa destructor*

Citation for published version:

Faber, N, Meiborg, Y, McFarlane, G, Gorjanc, G & Harpur, BA 2021 'A gene drive does not spread easily in populations of the honey bee parasite *Varroa destructor*' bioRxiv, at Cold Spring Harbor Laboratory.
<https://doi.org/10.1101/2021.04.30.442149>

Digital Object Identifier (DOI):

[10.1101/2021.04.30.442149](https://doi.org/10.1101/2021.04.30.442149)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



A gene drive does not spread easily in populations of the honey bee parasite *Varroa destructor*

Nicky R. Faber^{a,b,✉}, Adriaan B. Meiborg^a, Gus R. McFarlane^c, Gregor Gorjanc^a, and Brock A. Harpur^d

^aHighlanderLab, The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush Campus, Midlothian EH25 9RG, United Kingdom

^bLaboratory of Genetics, Department of Plant Sciences, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

^cBurdon Group, The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush Campus, Midlothian EH25 9RG, United Kingdom

^dDepartment of Entomology, Purdue University, West Lafayette, IN 47907, United States of America

1 **Varroa mites (*Varroa destructor*) are the most significant threat**
2 **to beekeeping worldwide. They are directly or indirectly re-**
3 **sponsible for millions of colony losses each year. Beekeepers**
4 **are somewhat able to control Varroa populations through the**
5 **use of physical and chemical treatments. However, these meth-**
6 **ods range in effectiveness, can harm honey bees, can be phys-**
7 **ically demanding on the beekeeper, and do not always provide**
8 **complete protection from Varroa. More importantly, in some**
9 **populations Varroa mites have developed resistance to available**
10 **acaricides. Overcoming the Varroa mite problem will require**
11 **novel and targeted treatment options. Here, we explore the po-**
12 **tential of gene drive technology to control Varroa. We show**
13 **that spreading a neutral gene drive in Varroa is possible but re-**
14 **quires specific colony-level management practices to overcome**
15 **the challenges of both inbreeding and haplodiploidy. Further-**
16 **more, continued treatment with acaricides is necessary to give a**
17 **gene drive time to fix in the Varroa population. Unfortunately, a**
18 **gene drive that impacts female or male fertility does not spread**
19 **in Varroa. Therefore, we suggest that the most promising way**
20 **forward is to use a gene drive which carries a toxin precursor or**
21 **removes acaricide resistance alleles.**

22 *Varroa destructor* | Gene drive | Genetic population control | Modelling
23 Correspondence: nfaber@outlook.com

24 Introduction

25 When the Varroa mite (*Varroa destructor*) jumped from its
26 original host the Eastern honey bee (*Apis cerana*) to the West-
27 ern honey bee (*Apis mellifera*), it spread rapidly around the
28 globe and caused catastrophic losses of commercial and feral
29 honey bee colonies (1–4). To this day, Varroa mites remain
30 the most highly-reported cause of colony loss for commercial
31 beekeepers and hobbyists (1, 5–7). There are treatment op-
32 tions available to beekeepers that allow them to control Var-
33 roa. Unfortunately, currently available treatments do not pro-
34 vide complete protection from Varroa and they often harm
35 honey bees or are physically demanding for the beekeeper.
36 For example, acaricides are among the most effective treat-
37 ments available and can kill between 49–82% of the Varroa
38 within a colony (8–10). Despite their effectiveness, some
39 acaricides also affect honey bees; they reduce honey bee fer-
40 tility (11), foraging, and immune responses against bacte-
41 rial infections (12). More concerning still, in some popu-
42 lations Varroa mites have developed resistance to acaricides

(13–16). Beyond chemical treatments, beekeepers can use
physical means of Varroa control such as drone brood re-
moval, which gives Varroa mites limited opportunities to re-
produce. However, physical methods can require significant
labour and thus may not be feasible on a large scale (17, 18).
The unfortunate fact of Varroa mite control is that it relies
on blunt chemical treatment methods that can harm bees and
may not be effective long-term because of evolved resistance.
This echoes similar treatment methods available to other pest
species around the globe like malarial-vectoring mosquitoes
and crop pests like spider mites (19–22).

Genetic population controls, like those that can be im-
plemented through the use of a gene drive (23), could be a
more successful and more sustainable means to control Var-
roa mites and other invertebrate pests than currently-available
chemical and physical methods (24). Gene drives are self-
ish genetic elements that can be engineered to promote the
inheritance of desired alleles at rates much greater than con-
ventional Mendelian inheritance (25). When a gene drive al-
lele is introduced into a population, it spreads through the
mating of gene drive carrying individuals with wild-type in-
dividuals (24). A CRISPR-based gene drive element encodes
the two components of CRISPR (a Cas nuclease and guide
RNA) and can contain a gene of interest one wishes to propa-
gate (26, 27), or it can be targeted to a gene one wants to dis-
rupt (28–30). In the germline of gene drive carriers, the Cas
nuclease and guide RNA are expressed to generate a double-
stranded DNA break on the opposing wild-type chromosome
at the gene drive locus. This DNA break is repaired through
homology-directed repair, using the gene drive harbouring
chromosome as the repair template, and thus the gene drive
element is copied to the second chromosome (24). The con-
version rates for gene drives in insects can be as high as 100%
(26, 31–33). This process occurs again in the offspring gen-
eration and will do so in all subsequent generations, resulting
in the gene drive spreading through the target population. A
gene drive can be designed to reduce the fitness of individual
homozygous carriers with the aim to reduce population size
or even achieve extirpation (23, 34).

The introduction of CRISPR-Cas9 gene drives as a man-
agement tool for Varroa numbers could greatly impact our
ability to control them, and technology is progressing to

85 a stage where we could test this strategy. The necessary
 86 biochemical and biological research is currently coming to-
 87 gether: *in vitro*-rearing techniques for Varroa are being re-
 88 fined (35, 36), there is a high-quality reference genome (37),
 89 and there is a growing list of genes essential to mite survival
 90 (38). CRISPR-Cas9-mediated mutagenesis has not yet been
 91 published for Varroa mites but recent work on spider mites
 92 demonstrates that this may soon be possible (39). However,
 93 we do not yet know if a gene drive can spread in a Varroa popu-
 94 lation. Prior to any gene drive system being implemented,
 95 it is essential to develop a species-specific genetic and demo-
 96 graphic model to predict the effectiveness of a drive spread-
 97 ing successfully (29, 30, 34, 40–44). This is especially im-
 98 portant in non-model species where mating biology and sex-
 99 determination systems can limit the spread of gene drives. In
 100 the case of Varroa mites, they can both outbreed and inbreed,
 101 and the proportion of each breeding strategy varies through-
 102 out the season based on brood cell availability (44, 45). In-
 103 breeding, along with haplodiploidy (46) in Varroa reduce the
 104 likelihood of a gene drive spreading effectively.

105 We present a modelling study to investigate the effective-

ness of a gene drive given the unique life history of Varroa. 106
 We estimate the spreading efficiency of a gene drive in a single 107
 honey bee colony and identify management techniques 108
 beekeepers may have to implement to successfully spread a 109
 gene drive in their colonies. We show that spreading a neutral 110
 gene drive in Varroa is challenging because of the high rate of 111
 inbreeding and their exponential growth rate that can quickly 112
 overwhelm a honey bee colony. Some management strategies, 113
 including the use of acaricides, may help spread gene 114
 drive alleles. Unfortunately, we could devise no scenario to 115
 spread gene drives that impact fitness traits like male or fe- 116
 male fertility. Therefore, we suggest that the most promising 117
 way forward is to use a gene drive which carries a toxin pre- 118
 cursor or removes acaricide resistance alleles. 119

Results 120

A. Development of a genetic population model of *Varroa destructor*. 121
 We first created a realistic, stochastic, popula- 122
 tion model of *Varroa destructor* that includes genetic inher- 123
 itance. For an overview and description of the model and life 124

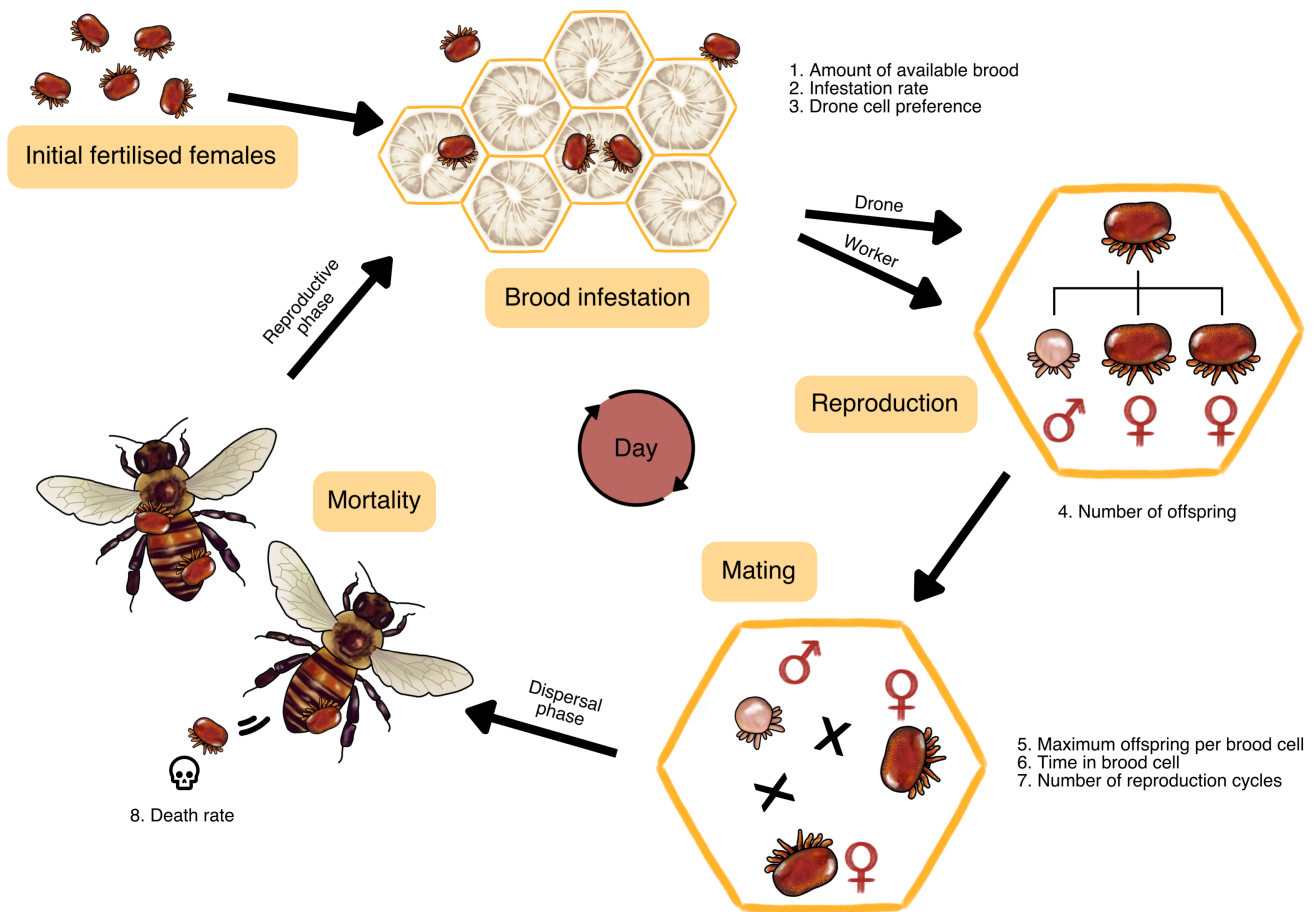


Figure 1. An overview of our Varroa demographic model. For full details, see the Methods section. First, we initialise a certain number of fertilised females. Then, we use a backbone model of an average honey bee colony in a temperate climate where a certain amount of new brood cells become available for Varroa infestation every day. The Varroa infest these cells at a certain rate depending on the number of brood cells and adult bees. Varroa prefer drone cells over worker cells, because those are capped for 2 days longer (14 instead of 12 days), which enables more Varroa offspring to mature. Once in the cell, the fertilised females lay 1 male offspring followed by a varying number of female offspring. Once the females mature, they mate with the male. We assign each female a certain number of reproduction cycles, so one Varroa female can infest brood cells multiple times throughout her life. Then, the fully grown bee emerges from the cell with the Varroa attached to them, which is the start of the Varroa's dispersal phase. At this stage we model a certain mortality rate which accounts for all ways in which a Varroa could have died during its life cycle.

125 history parameters, see Figure 1 and Methods. Our model has
126 a population trajectory that is similar both in shape and am-
127 plitude to previous modeling (47–49) and empirical studies
128 (50) (Figure 2A). The model begins on day 1 of the calend-
129 ar year, a period of low or no growth for temperate popula-
130 tions. The population steadily declines due to daily mortal-
131 ity. By the summer, the Varroa population grows exponen-
132 tially. The starting population of Varroa greatly influences
133 the speed with which Varroa reach threshold levels within
134 a colony. With 100, 10, or 1 initial Varroa, it respectively
135 takes one, two, or three years longer for the population to
136 reach the threshold of 10,000 individuals where we stop our
137 model. The level of Varroa infestation at which beekeepers
138 will typically treat colonies is reached a year earlier. With 1
139 initial Varroa, this single Varroa often dies in the winter and
140 therefore, the population grows in only a small number of
141 replicates. Importantly, we observe more variability in mod-
142 els that begin with fewer Varroa. This variability is caused by
143 the timing of reproduction of few Varroa, where small initial
144 differences will grow bigger with the exponential growth.

145 We were also able to quantify the seasonal fluctuations in
146 inbreeding in our modelled population (Figure 2B). We es-
147 timated the mean homozygosity at 1000 bi-allelic loci (with
148 an initial average allele frequency of 0.5) across a single re-
149 combining chromosome. We began each model with a mean
150 homozygosity at the beginning of the year of 0.95 in line with
151 previous estimates for Varroa (51). We found that homozy-
152 gosity remains high throughout most of the beekeeping sea-
153 son but there are pronounced drops in homozygosity during
154 the end of a typical year. This represents a period of time
155 when honey bee colonies are reducing brood production and
156 Varroa populations are typically high. This combination in-
157 creases the amount of mated Varroa sharing cells, increases
158 the chance of their offspring outbreeding, and thus reducing
159 homozygosity. Overall, our model is qualitatively similar to
160 expectations for a typical Varroa population in a managed
161 honey bee colony living in a temperate climate.

162 **B. Inbreeding hinders gene drive spread and a fit-
163 ness-affecting gene drive cannot spread.** We model the
164 release of 1 homozygous gene drive carrying Varroa into a
165 population of 10 wild-type Varroa (gene drive frequency of
166 0.09), which is relatively high for a non-threshold dependent
167 gene drive (42, 52). We then track the genotypes and allele
168 frequencies of individual Varroa in a single honey bee colony
169 (Figure 2C, D). As can be seen in both plots, the wild-type al-
170 lele and wild-type genotypes remain the most prevalent even
171 if we allow the model to continue to a population size of
172 10,000 Varroa mites, greatly exceeding population sizes ob-
173 served in typical colonies (53). Our model strongly suggests
174 that typical gene drive release frequencies may not be suffi-
175 cient to spread a gene drive in Varroa. This is likely a result
176 of inbreeding, given that gene drive homozygotes are more
177 prevalent than gene drive heterozygotes over the course of
178 the simulation (Figure 2C). As well, gene drive alleles only
179 meaningfully increase in the last days of the model when Var-
180 roa numbers are high and cell sharing increases. The dy-
181 namics described above are consistent even when increasing

182 the initial population size and released gene drive individuals
183 (Figure S1). We found that our model is not sensitive to pa-
184 rameters influencing the spread of gene drive alleles (Figure
185 S2). In the context of population control, the goal of a gene
186 drive is to reduce population sizes by spreading alleles that
187 reduce fitness. We could not conceive a model that success-
188 fully spread a male- or female-specific fitness-reducing drive
189 (Figure S3).

190 **C. With high introduction frequencies, a gene drive
191 approaches fixation.** When Varroa numbers are still low at
192 the start of the year, it is possible to introduce a larger amount
193 of gene drive Varroa to immediately obtain a high gene drive
194 allele frequency. More importantly, this higher gene drive
195 allele frequency could ensure that whenever outbreeding oc-
196 curs, a gene drive Varroa is likely involved. Therefore, we
197 modelled a population of 10 wild-type Varroa with either
198 1, 10, or 50 added homozygous gene drive Varroa. These
199 amounts respectively give initial gene drive frequencies of
200 0.09, 0.50, and 0.83. We find that the gene drive allele in-
201 creases most rapidly at an initial release frequency of 0.5,
202 because an outbreeding event is most likely between a gene
203 drive Varroa and a wild-type Varroa, rather than between two
204 wild-types or between two gene drives (see Figure 3 and Fig-
205 ure S4). Naturally, a high initial gene drive frequency re-
206 sults in the highest gene drive allele frequency in the end.
207 Therefore, a high initial release frequency might be benefi-
208 cial to spread a gene drive through a Varroa population.
209 Unfortunately, we also see that with an initial amount of 50
210 gene drive Varroa, the population reaches 10,000 individuals
211 a year sooner than with 1 or 10 added Varroa (see Figure 3).

212 **D. Brood breaks increase outbreeding, but do not
213 meaningfully increase the spread of a gene drive.**
214 Above, we demonstrate that outbreeding can be impacted by
215 the initial release frequency of gene drive Varroa. Ultimately,
216 the amount of cell sharing, and thus outbreeding, depends on
217 three factors: the amount of Varroa, the amount of available
218 brood, and the amount of adult honey bees (54). Therefore,
219 decreasing the number of available honey bee brood cells
220 can increase outbreeding frequency. Cell availability typi-
221 cally decreases naturally at the end of a beekeeping season
222 when honey bees reduce egg laying. Beekeepers can also ar-
223 tificially change cell availability by preventing or restricting
224 queens from laying eggs, a period called a 'brood break' (17).

225 We tested two brood break strategies for their effective-
226 ness at increasing outbreeding and the fixation rate of gene
227 drive alleles. For the first strategy we entirely stopped brood
228 production, forcing Varroa to stay in the dispersal phase (left-
229 most column in Figure 4). After this brood break, Varroa
230 would more likely infest newly available brood with multi-
231 ple Varroa per cell. For the second strategy, we provided a
232 steady but lowered amount of brood throughout the brood
233 break (middle three columns in Figure 4). We also modelled
234 no brood break intervention as a control (right-most column
235 in Figure 4). For each of these strategies, we modelled three
236 different brood break starting days: 110 (early season, when
237 brood production is just starting), 160 (middle season, when

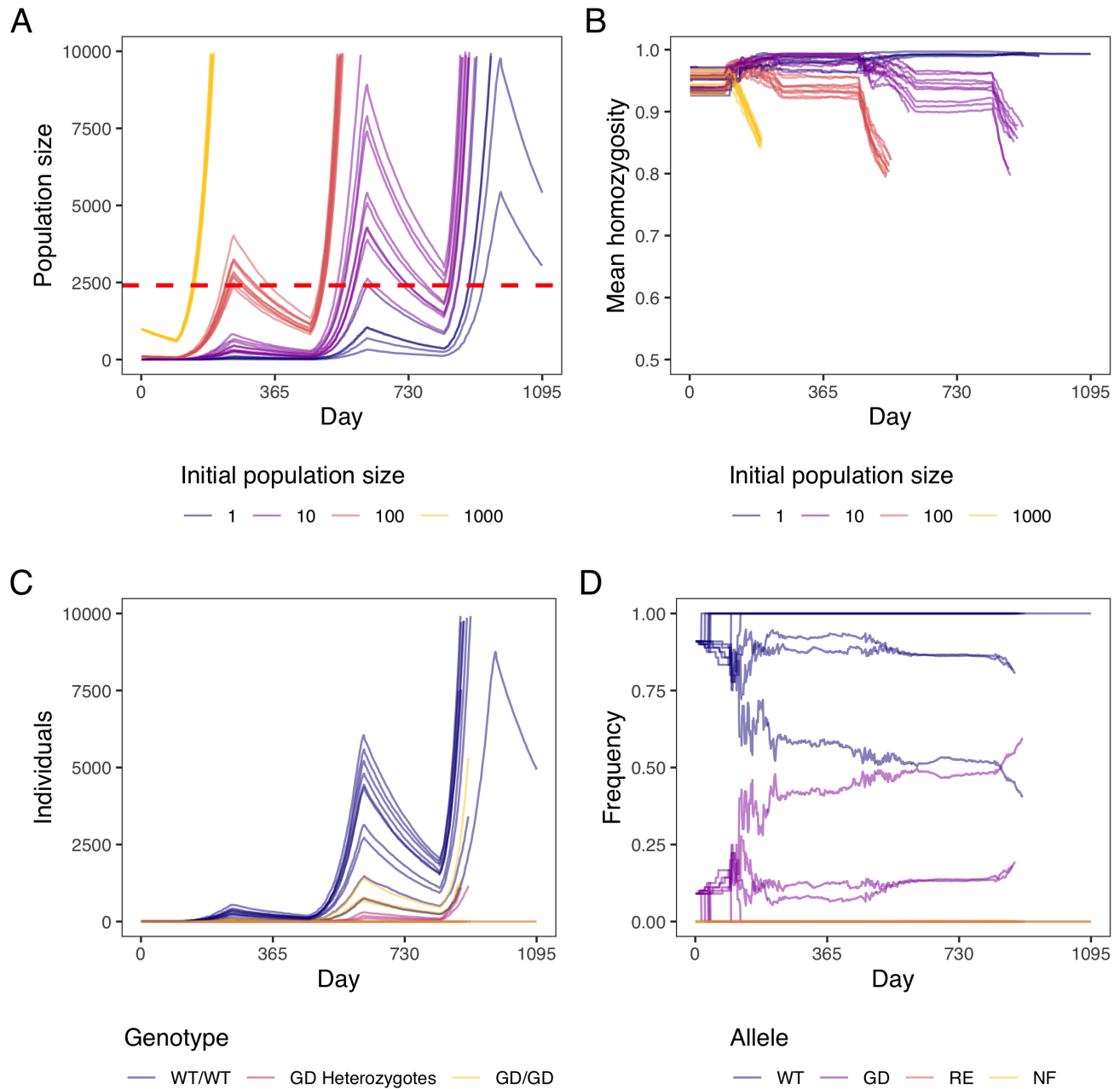


Figure 2. Model of Varroa and gene drive spread. For every set of parameters, we run 10 repetitions and stop the model when the Varroa population size is over 10,000. **A)** Population size over three years with different initial population sizes. The dashed red line indicates a Varroa prevalence of 5% in summer (5 Varroa per 100 adult bees), which is used by beekeepers as a "danger threshold" where treatment is necessary for bee colony health. **B)** Mean homozygosity over three years with different initial population sizes. We model a single chromosome with 1000 bi-allelic loci, each with initial average frequency of 0.5. We initiate individuals at 95% homozygosity because Varroa have very high inbreeding coefficients of 0.9. **C)** Numbers of individuals with three genotypes over three years: WT = wild-type and GD = gene drive. The initial population size was 10 wild-type Varroa with 1 added homozygous gene drive Varroa. **D)** Frequencies of gene drive alleles over three years: WT = wild-type, GD = gene drive, RE = resistant, and NF = non-functional. The initial population size was 10 wild-type Varroa with 1 added homozygous gene drive Varroa, giving an initial gene drive frequency of 0.09.

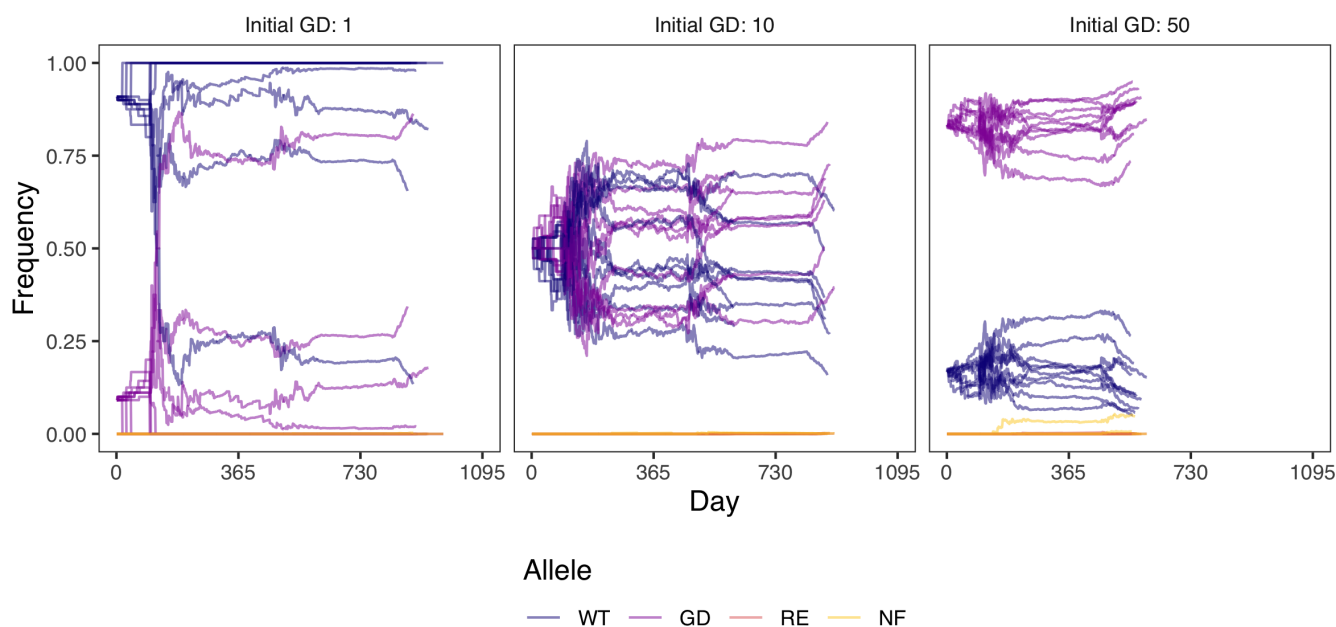


Figure 3. Allele frequencies over three years with different gene drive introductions. The initial population size is 10 wild-type Varroa with 1, 10 or 50 added homozygous gene drive Varroa, giving respective initial gene drive frequencies of 0.09, 0.50, and 0.83. WT = wild-type, GD = gene drive, RE = resistant, and NF = non-functional. For every set of parameters, we run 10 repetitions and stop the model when the Varroa population size is over 10,000.

brood production is at its maximum), and 210 (late season, just before brood production stops). Both strategies increased the amount of cell sharing (see Figure S6). However, only the strategy where a beekeeper adds in a specific proportion of brood during the break increased the the frequency of heterozygous gene drive Varroa in a colony relative to the control without brood break (see Figure 4). A brood break with a beekeeper allowing between 0.01 - 0.1 of available cells to be used for brood was the most effective. In practice, this equates to approximately one full frame in a ten-frame Langstroth colony. These results suggest that with some fine-tuning, outbreeding can be increased by the beekeeper and therefore increasing the likelihood of fixing a gene drive.

Gene drive allele frequency should increase after heterozygotes produce offspring, as gene drive homing will occur in these individuals. Thus, during a brood break, we first expect an increase in heterozygotes as outbreeding occurs, followed by an increase in gene drive allele frequency as these heterozygotes reproduce. However, we show in Figure S7 that there is only a modest increase in gene drive allele frequency after the brood break compared to no brood break. This is likely because of the low frequency of heterozygotes, which is lower than 0.2 as can be seen in Figure 4. In this model, we added the same amount of gene drive Varroa as there are wild-type Varroa, so the allele frequencies are both 0.5. As we showed in Figure 3, this ratio leads to the most rapid increase in gene drive allele frequency. Indeed, in Figure S8 where we model a larger gene drive introduction frequency, the frequency of gene drive heterozygotes is even lower. Despite the high introduction frequency and brood breaks, the gene drive is still not able to fix in the population (see Figure S9). These results show that brood breaks are

unlikely to have a large effect on the spread of a gene drive.

E. Acaricide treatment may facilitate gene drive fixation. None of the scenarios we ran were able to fix a gene drive before Varroa reached threshold levels within a honey bee colony. To that end, we incorporated an acaricide treatment into the model that would be activated anytime a colony reached threshold Varroa levels (Figure 5). We found that effective acaricide treatments provide additional time for a gene drive to reach fixation. However, acaricide treatments significantly increase the variability between the model repetitions, which does not disappear when starting the model with a higher number of initial Varroa (Figure S10). This means that the observed variability is due to the fact that, by chance, we could be removing more gene drive Varroa than wild-types. Therefore, gene drive fixation is not reached very fast and not in all populations.

The best acaricide strategy for gene drive fixation was with 80% acaricide effectivity. With this effectivity Varroa populations reach the treatment threshold multiple times within a single year and multiple acaricide treatments are necessary. These repeated relatively ineffective treatments are less prone to variability but probably not desirable in practice. We show that introducing more gene drive carriers after acaricide treatment facilitates faster gene drive fixation and less variability (see Figure S11). At this point gene drive fixation is probably due to population replacement rather than gene drive spread.

Discussion

The greatest threat to managed honey bee colonies, globally, is the Varroa mite (1, 5–7). With the ever-advancing toolkit

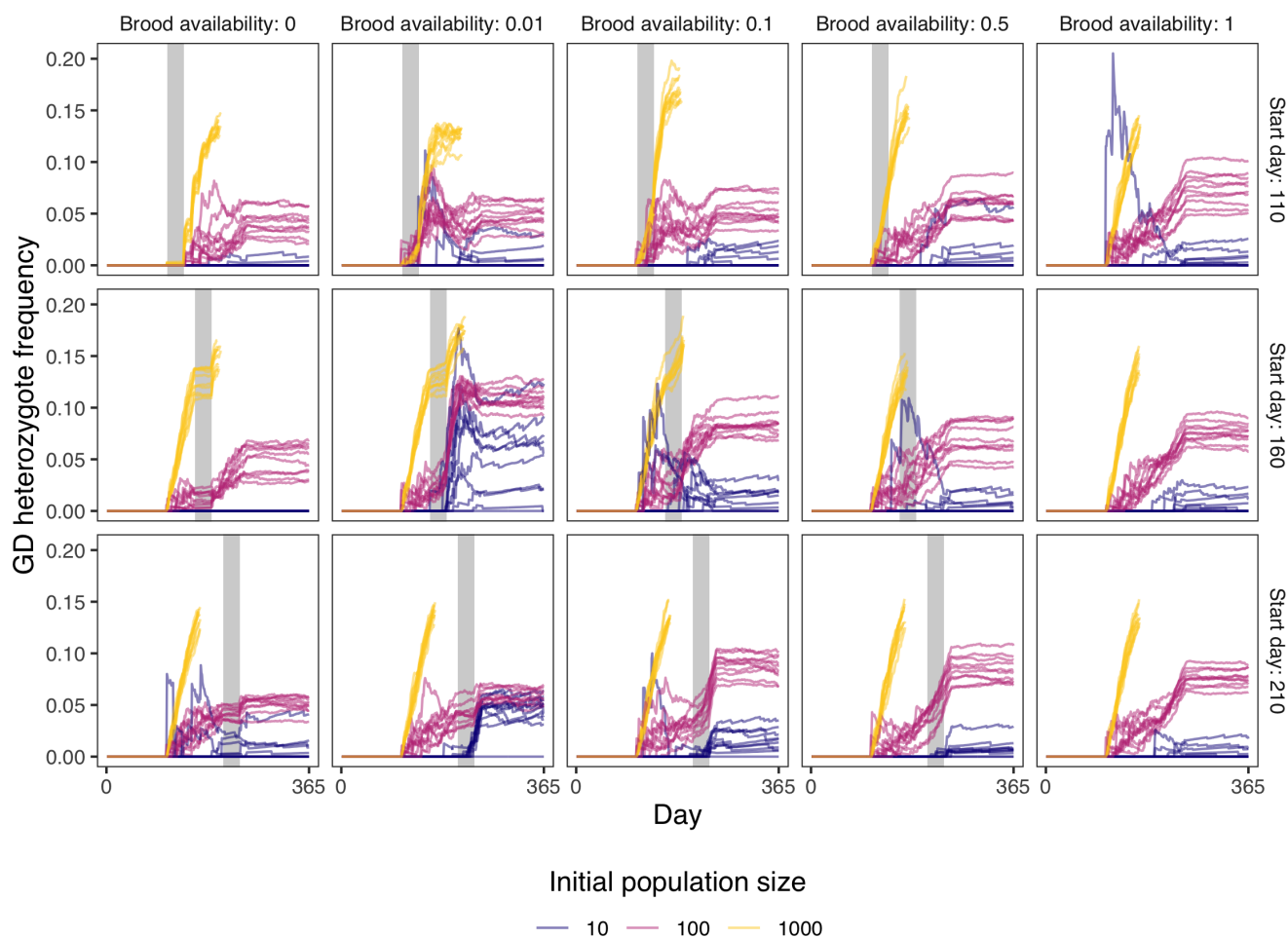


Figure 4. Gene drive (GD) heterozygote frequency over time for different initial population sizes, given different amounts of brood cell availability (as a fraction of the normal amount) and different brood break starting days. The grey bars indicate the brood break. The initial population sizes were 10, 100, or 1000 wild-type Varroa with the same number of gene drive Varroa on top of that, giving an initial gene drive frequency of 0.5. For every set of parameters, we run 10 repetitions and stop the model when the Varroa population size is over 10,000.

300 available to study functional genomics in Varroa (37, 55, 56),
 301 we suggest that the prospect of genetic control is not far from
 302 a reality. We set out to test the feasibility of such a system, in
 303 the form of a gene drive, in a modelling study of a population
 304 of Varroa within a single honey bee colony. We demonstrate
 305 that a neutral gene drive could spread in a Varroa population
 306 in a honey bee colony and open the door to future analysis
 307 in exploring how to spread gene drives in non-model species
 308 with particularly challenging biology.

309 A gene drive could work in Varroa but it is slow and
 310 requires management inputs. Our stochastic model tracked
 311 the growth of Varroa mite populations each day over several
 312 years in a typical temperate honey bee colony. Varroa living
 313 in colonies in non-temperate climates will likely need addi-
 314 tional modelling given the very different demography that
 315 honey bees have in these areas (57). We focused on temper-
 316 ate colonies, specifically, because they represent most man-
 317 aged colonies in the United States (5) and because temper-
 318 ate climates provide an opportunity for increased outbreeding
 319 in Varroa. Varroa populations tend to be highest in the fall
 320 (47, 58, 59). During this time, honey bee colonies decrease

brood production to prepare for the winter. As we observe
 and others have empirically demonstrated, Varroa mites in-
 crease outbreeding rates in the fall because of reduced brood
 cell availability (51). Outbreeding is critical to the establish-
 ment of a Varroa gene drive and indeed to any gene drive
 (45).

We could not conceive a model that would successfully
 spread a lethal gene drive in Varroa. The most promis-
 ing way forward may be to design neutral drives with envi-
 ronmentally-induced fitness effects (such as the spread-
 ing a toxin precursor), drives which remove acaricide resis-
 tance alleles, or drives that target genes involved in Varroa-
 viral interactions. Each of these requires a deeper under-
 standing of Varroa functional genomics but may be fruit-
 ful for future investigations. Spreading drives that confer
 Varroa with genetic resistance against viruses is a particu-
 larly interesting prospect. The threat that Varroa mites pose
 to honey bees is exacerbated by the viruses they introduce
 into their hosts (60–62).

There are several challenges to establishing a gene drive
 in Varroa that need to be overcome. Natural outbreeding

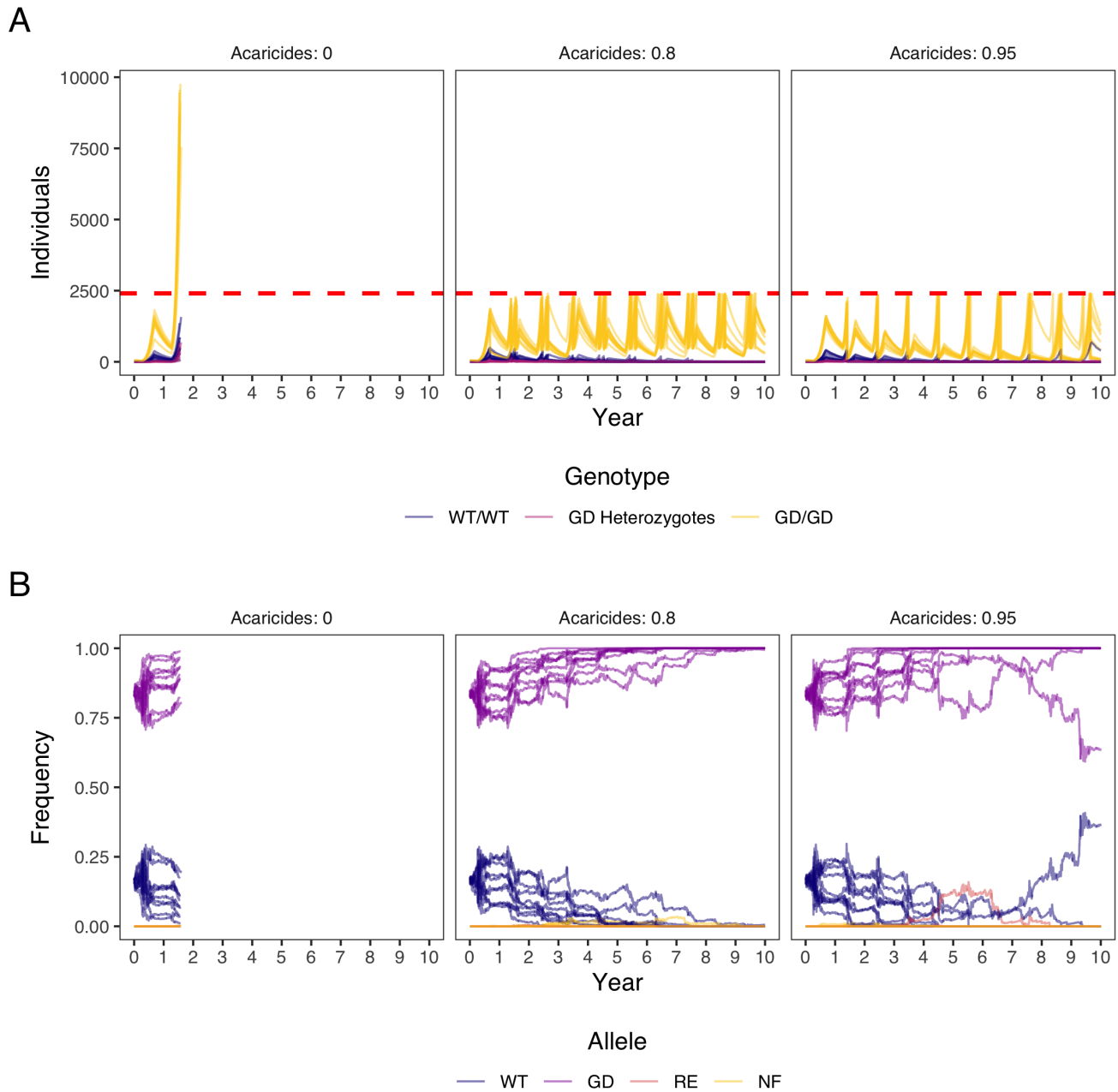


Figure 5. The spread of a gene drive while the Varroa population is suppressed with acaricides whenever the Varroa prevalence surpasses the danger threshold of 5% in summer (5 Varroa per 100 adult bees). The initial population size was 10 wild-type Varroa with 50 homozygous gene drive Varroa, giving an initial gene drive frequency of 0.83. For every set of parameters, we run 10 repetitions and stop the model when the Varroa population size is over 10,000. **A)** Frequencies of gene drive genotypes over time, given different intensities of acaricide treatment when the population surpasses the danger threshold. WT = wild-type, GD = gene drive. **B)** Frequencies of gene drive alleles over time, given different intensities of acaricide treatment when the population surpasses the danger threshold. WT = wild-type, GD = gene drive, RE = resistant, and NF = non-functional.

342 alone was not enough to reliably increase the frequency of
343 gene drive. We attempted to overcome this challenge by
344 incorporating beekeeper management in the form of brood
345 breaks and acaricide treatments. Both influenced the rate of
346 outbreeding and the likelihood of gene drive fixation. Im-
347 portantly, both of these management practices are used by
348 beekeepers and their incorporation into future gene drive ef-
349 forts would not be an additional burden. The need for bee-
350 keeper management also suggests that a drive has a limited
351 ability to spread beyond the apiary. All gene drive mod-
352 els we attempted faced the additional challenge of concomi-
353 tantly minimizing population growth. When Varroa popula-
354 tions exceed economic thresholds, honey bee colonies pro-
355 duce less honey and have a higher probability of collapsing
356 (63, 64). Here, we took a very generous threshold of 5 Var-
357 roa/100 bees across the year and ran simulations until Varroa
358 reached 10,000 mites in a single colony — a level that would
359 almost never be observed in a managed colony. Furthermore,
360 because Varroa populations grow exponentially, a honey bee
361 colony can only go without Varroa control for a few years
362 at most, depending on the initial infestation level. Control-
363 ling Varroa growth with acaricides was an effective means
364 to improve the spread of neutral gene drives by providing
365 more time for the gene drives to fix before the honey bee
366 colony reached 10,000 Varroa. However, this method in it-
367 self is troubling because it does not remove the risk of Varroa
368 populations evolving acaricide resistance nor does it remove
369 the risk that some acaricides pose to honey bees. We feel
370 that the addition of management scenarios in our models and
371 others (30) is particularly important for the gene drive litera-
372 ture and a feature that could be overlooked. Incorporating the
373 typical management practices into models and understanding
374 how they impact gene drive dynamics may be an important
375 addition to future work.

376 In summary, our models provide an early look at how
377 gene drives may act in the Varroa system. They are by
378 no means comprehensive. Varroa occupy a huge range and
379 experience different colony and apiary environments across
380 it. Location- or management-specific models may reveal
381 that gene drives spread more or less successfully. The genetic
382 background of a honey bee colony and a colony's response to
383 increasing Varroa loads were also not modelled. Both could
384 impact the spread of a gene drive. The population dynam-
385 ics for Varroa in Varroa-tolerant or resistant colonies is likely
386 different and could impact the spread of a gene drive, per-
387 haps acting like acaricide treatments and providing a longer
388 time for gene drives to spread. Any colony-level responses
389 to increased levels of Varroa parasitism could increase or de-
390 crease the likelihood of a drive spreading. We also did not ex-
391 plore dynamics outside of a single honey bee colony and did
392 not explore the risks of modified Varroa establishing in non-
393 target colonies. Varroa mites are as highly mobile as honey
394 bees and more modelling is necessary to understand the roles
395 of drifting, foraging, robbing, and management in spreading
396 gene drives outside of target colonies (65–68). We suggest,
397 given the difficulty we found in spreading drives in a single
398 colony, that the above factors may be unlikely to establish

399 drives in non-target colonies. Even if they could establish
400 outside of target colonies, the spread of gene drive Varroa
401 may not be viewed as a major threat, at least in North Amer-
402 ica. This may not be the case in other parts of its introduced
403 range. In its native range, *Varroa destructor* can be found in
404 low frequency in *Apis cerana* colonies where we have little
405 information about its native ecology.

406 To our knowledge, genetic modification has not been per-
407 formed in Varroa mites and *in vitro* rearing methods are, so
408 far, unable to maintain a breeding population of Varroa (55).
409 Mutagenesis in chelicerates has recently been accomplished
410 (39) but transgenesis has yet to be achieved. Gene drives may
411 be many years off for Varroa. With more expertise develop-
412 ing in the fields of transgenesis and mutagenesis in arthro-
413 pods, it is likely that we will see experiments in the Varroa
414 system and we hope that our work can help develop ideas
415 about genetic control of this invasive pest species. In the
416 short-term, currently-available treatment methods (63) and
417 perhaps newer methods (38, 69) remain the best methods to
418 control Varroa.

419 Methods

420 Within R 4.0.5 (70), we used the package AlphaSimR as a
421 framework for our modelling (71). AlphaSimR is designed
422 to model the genetics of plant and animal breeding schemes,
423 but lends itself well to general population genetics modelling
424 too. We have created an individual-based, stochastic, day-
425 by-day model of *Varroa destructor* (hereafter simply named
426 Varroa), which consist of three aspects: a static honey bee
427 colony as backbone, a stochastic model of Varroa and its life
428 history, and the implementation of a gene drive. Everyday in
429 the model, we track parameters such as the size of the Varroa
430 population, the levels of inbreeding, and the allele frequen-
431 cies at the gene drive locus, among others.

432 **A. Honey bee colony simulation.** Varroa is a parasite and
433 depends on its host, *Apis mellifera*, for reproduction. There-
434 fore, to realistically model a population of Varroa, we must
435 also model a honey bee colony. We chose to use a static
436 model for the honey bee colony, as we are primarily inter-
437 ested in the Varroa population and not the interaction between
438 parasite and host. We used a honey bee colony model from
439 Calis et al. (1999) (48), who based their model on data from
440 Allen (1965) (72). This model is based on a colony of average
441 size in a Northern European climate and contains the amount
442 of adult honey bees, drone brood, and worker brood over 365
443 days. At the end of the year, bee and brood numbers are the
444 same as at the start of the year. Therefore, we can model mul-
445 tiple years by replicating this honey bee model several times
446 back to back. We assumed that a honey bee colony would col-
447 lapse when the Varroa population reaches 10,000 individuals,
448 at which point we stopped the model. We also implemented
449 an option to reduce brood amounts through colony manage-
450 ment by the beekeeper to manage inbreeding in the Varroa
451 population (73). For a variable amount of days, we reduce
452 the brood by a variable percentage of its original amount on
453 those days. In our fixed honey bee colony model, we only

454 change the amount of drone and worker brood and leave the
455 adult bee numbers the same.

456 **B. Varroa life history.** Our model consists of a number of
457 steps to accurately represent the complex life history of Var-
458 roa mites:

459 1. **Initialising mated females.** At the start of the model,
460 we initialise a certain number of mated Varroa females.
461 Then, every time when female Varroa offspring is cre-
462 ated, we assign each Varroa a certain number of repro-
463 duction cycles it will go through in its life. Current
464 estimates of how many reproduction cycles are com-
465 pleted on average range between 2 to 3 (74, 75). There-
466 fore, we assign each female a number between 1 and 4
467 randomly, which gives an average of 2.5 reproduction
468 cycles.

469 2. **Brood infestation.** The first step in Varroa reproduc-
470 tion is the infestation of a honey bee brood cell. For
471 the rate of brood entering, we use a model by Boot et
472 al. (1994) (54), who tested several models to predict
473 this rate. On every day of our model, we calculate the
474 number of infestations (N_i) as:

$$N_i = 1 + e^{-(2.87 + 0.00385 * \frac{N_b}{N_a} * 10000)}^{-1}, \quad (1)$$

475 which is dependent on the ratio between available
476 brood (N_b) and the number of adult bees (N_a) (54).
477 The biological reasoning behind this model is that Var-
478 roas are phoretic on adults bees and when those bees
479 get close to available brood cells, the Varroa can in-
480 fest (54). When this ratio is low, the probability that an
481 adult bee with a phoretic Varroa will pass by an avail-
482 able brood cell is low, and vice versa.

483 Once we have determined the number of Varroa that in-
484 fest, we assign them to the available drone and worker
485 cells. Varroa prefer drone cells over worker cells, be-
486 cause those are capped for 2 days longer (14 instead of
487 12 days) (47), which enables more Varroa offspring to
488 mature. We model a drone cell preference by giving
489 drone cells an eight times higher probability of infes-
490 tation (76). Therefore, by chance any drone or worker
491 cell could be infested by more than one Varroa, with
492 the probability of this happening being much higher in
493 drone cells.

494 3. **Generating offspring.** Varroa mites first produce a
495 single male offspring, followed by a varying number
496 of female offspring (1). More female offspring are able
497 to mature in drone brood than in worker brood because
498 of the longer capping period of those cells (77). There-
499 fore, we use two separate distributions to determine the
500 number of female offspring per Varroa in the two types
501 of brood as described by Infantidis (1984) (59). These
502 distributions include Varroa that produce no offspring
503 as well. The averages of these distributions for female
504 offspring are 1.70 for drone cells and 0.71 for worker

505 cells (59). Excluding the non-productive Varroa, the
506 averages of female offspring are 2.77 for drone cells
507 and 1.33 for worker cells (59).

508 4. **Mating between offspring.** Varroa offspring mate in
509 the brood cell they are born in (78). Usually only one
510 Varroa infests a cell, which forces offspring to inbreed
511 by full-sibling mating. Occasionally however, espe-
512 cially at the end of the season when Varroa numbers
513 are high, multiple Varroa infest a single cell, which al-
514 lows for outbreeding (51). Mated females will gener-
515 ate offspring the rest of their lives with the sperm they
516 save in their spermatheca (77). We model random mat-
517 ing between males and females in a brood cell, where
518 females mate with a single male.

519 5. **Emergence from brood.** In every brood cell, there is
520 a limit to how many Varroa offspring can survive (79).
521 According to data from Martin (1995) (79), the max-
522 imum live offspring per cell is 16 in drone cells and
523 8 in worker cells. Additionally, they show that there
524 is usually one male offspring for every mother mite,
525 so mostly female offspring will not survive in over-
526 crowded brood. This is likely because of competition
527 at the feeding site (79). Therefore, we determine the fe-
528 male offspring survival probability (P_s) per brood cell:

$$P_s = \begin{cases} 0 & f > max - m \\ 1 - \frac{max - m}{f} & f \leq max - m \end{cases}, \quad (2)$$

529 where (m) is the number of male offspring, (f) the
530 number of female offspring, and (max) the maximum
531 number of offspring in that type of brood.

532 6. **Mortality.** In our model, we expect 0.5% of Varroa to
533 die every day, which is the average between the sum-
534 mer and winter mortality used by Fries et al. (1994)
535 (47). Additionally, we remove Varroa who have gone
536 through their final reproduction cycle, after which they
537 are assumed to die (74).

538 **C. Gene drive implementation.** Although AlphaSimR
539 was designed to model large numbers of loci for breeding and
540 quantitative genetics, the framework is perfect for the single
541 locus of a gene drive too. Each individual is modelled with a
542 single gene drive locus on two chromosomes and inheritance
543 is random.

544 We have implemented a gene drive which homes in the
545 germline and has four potential alleles: wild-type, gene drive,
546 resistance, and non-functional. Like Prowse et al. (2017)
547 (42), we model a probability of cutting (P_C) of 0.95, a
548 probability of non-homologous end joining (P_{NHEJ}), which
549 is variable, a probability that non-functional repair occurs
550 (P_{NFR}) of 0.67, which is the probability of a frame-shift
551 occurring.

552 ACKNOWLEDGEMENTS

553 G.G. acknowledges support from the BBSRC to The Roslin Institute
554 (BBS/E/D/30002275) and The University of Edinburgh's Data-Driven Innovation

555 Chancellor's fellowship. B.A.H. was supported by Purdue University and funding
556 from Project Apis M.

AUTHOR CONTRIBUTIONS

557 G.G. and B.A.H. conceived the Varroa gene drive project. N.R.F. conducted
558 the modelling with assistance from A.B.M. and G.G.. B.A.H. guided the Varroa life
559 history aspects of the project, and G.R.M. and N.R.F. guided the gene drive aspects.
560 N.R.F. and B.A.H. wrote the manuscript and all authors reviewed it.
561

DATA AVAILABILITY

562 Our model code and data can be found on the HighlanderLab GitHub:
563 https://github.com/HighlanderLab/nfaber_varroa_gd.
564

COMPETING INTERESTS

565 The authors declare no competing interests.
566

References

1. Kirsten S Traynor, Fanny Mondet, Joachim R de Miranda, Maeva Techer, Vienna Kowalik, Melissa AY Oddie, Panuwan Chantawannakul, and Alison McAfee. Varroa destructor: A complex parasite, crippling honey bees worldwide. *Trends in Parasitology*, 2020.
2. Stephen L Buchmann and Gary Paul Nabhan. The pollination crisis: the plight of the honey bee and the decline of other pollinators imperils future harvests. *Sciences*, 36:22+, 1996.
3. Adrian M Wenner, W W Bushing, and Others. Varroa mite spread in the united states. *Bee Culture*, 124(6):341–343, 1996.
4. Bernhard Kraus and Robert E Page. Effect of varroa jacobsoni (mesostigmata: Varroidae) on feral apis mellifera (hymenoptera: Apidae) in california. *Environ. Entomol.*, 24(6):1473–1480, December 1995.
5. Kelly Kulhanek, Nathalie Steinhauer, Karen Rennich, Dewey M Caron, Ramesh R Sagili, Jeff S Pettis, James D Ellis, Michael E Wilson, James T Wilkes, David R Tarp, Robyn Rose, Kathleen Lee, Juliana Rangel, and Dennis vanEngelsdorp. A national survey of managed honey bee 2015–2016 annual colony losses in the USA. *null*, 56(4):328–340, August 2017.
6. Dennis vanEngelsdorp and Marina Doris Meixner. A historical review of managed honey bee populations in europe and the united states and the factors that may affect them. *J. Invertebr. Pathol.*, 2010.
7. Ana Molineri, Agostina Giacobino, Adriana Pacini, Natalia Bulacio Cagnolo, Julieta Merke, Emanuel Orellano, Ezequiel Bertozzi, Luis Zago, Andrea Aignasse, Hernán Pietronave, et al. Environment and varroa destructor management as determinant of colony losses in apiaries under temperate and subtropical climate. *Journal of Apicultural Research*, 57(4):551–564, 2018.
8. Marco Pietropaoli and Giovanni Formato. Acaricide efficacy and honey bee toxicity of three new formic acid-based products to control varroa destructor. *Journal of Apicultural Research*, 58(5):824–830, 2019. doi: <https://doi.org/10.1080/00218839.2019.1656788>.
9. Gerardo Pérez Santiago, Gabriel Otero-Colina, David Mota Sánchez, Martha Elva Ramírez Guzmán, and Rémy Vandame. Comparing effects of three acaricides on varroa jacobsoni (acar: Varroidae) and apis mellifera (hymenoptera: Apidae) using two application techniques. *Fla. Entomol.*, 83(4):468–476, 2000.
10. Morgan A Roth, James M Wilson, Keith R Tignor, and Aaron D Gross. Biology and management of varroa destructor (mesostigmata: Varroidae) in apis mellifera (hymenoptera: Apidae) colonies. *J. Integr Pest Manag.*, 11(1), January 2020.
11. Juliana Rangel and Adrian Fisher. Factors affecting the reproductive health of honey bee (apis mellifera) drones—a review. *Apidologie*, September 2019.
12. Hanan A Gashout, Ernesto Guzman-Novoa, and Paul H Goodwin. Synthetic and natural acaricides impair hygienic and foraging behaviors of honey bees. *Apidologie*, August 2020.
13. Diana Sammataro, Pia Untalan, Felix Guerrero, and Jennifer Finley. The resistance of varroa mites (acar: Varroidae) to acaricides and the presence of esterase. *null*, 31(1):67–74, March 2005.
14. P J Elzen, D Westervelt, and Others. Detection of coumaphos resistance in varroa destructor in florida. *Am. Bee. J.*, 142(4):291–292, 2002.
15. Patti J Elzen, James R Baxter, Marla Spivak, and William T Wilson. Control of varroa jacobsoni oud. resistant to fluralinate and amitraz using coumaphos. *Apidologie*, 31(3):437–441, 2000.
16. Norberto Milani. The resistance of varroa jacobsoni oud. to acaricides. *Apidologie*, 30(2-3):229–234, 1999.
17. N W Calderone. Evaluation of drone brood removal for management of varroa destructor (acar: Varroidae) in colonies of apis mellifera (hymenoptera: Apidae) in the northeastern united states. *J. Econ. Entomol.*, 98(3):645–650, June 2005.
18. Nicholas P Aliano and Marion D Ellis. A strategy for using powdered sugar to reduce varroa populations in honey bee colonies. *null*, 44(2):54–57, January 2005.
19. Rachel Carson. *Silent Spring*. Houghton Mifflin Harcourt, 1962.
20. Chusak Prasittisuk and James R Busvine. DDT-resistant mosquito strains with cross-resistance to pyrethroids. *Pestic. Sci.*, 8(5):527–533, October 1977.
21. Howard Baker. Spider mites, insects and DDT. *Yearbook of Agriculture*, 1952:562–566, 1952.
22. Timothy J Dennehy, Jeffrey Granett, and Thomas F Leigh. Relevance of Slide-Dip and residual bioassay comparisons to detection of resistance in spider mites. *J. Econ. Entomol.*, 76(6):1225–1230, December 1983.
23. Jackson Chamber, Anna Buchman, and Omar S Akbari. Cheating evolution: engineering gene drives to manipulate the fate of wild populations. *Nature Reviews Genetics*, 17(3):146, 2016. doi: <https://www.doi.org/10.1038/nrg.2015.34>.
24. Kevin M Esvelt, Andrea L Smidler, Flaminia Catteruccia, and George M Church. Emerging technology: concerning rna-guided gene drives for the alteration of wild populations. *Elife*, 3:e03401, 2014.
25. Gus R McFarlane, C Bruce A Whitelaw, and Simon G Lillico. Crisp-based gene drives for pest control. *Trends in biotechnology*, 36(2):130–133, 2018.
26. Valentino M Gantz, Nijole Jasinskiene, Olga Tatarenkova, Aniko Fazekas, Vanessa M Macias, Ethan Bier, and Anthony A James. Highly efficient cas9-mediated gene drive for population modification of the malaria vector mosquito anopheles stephensi. *Proc. Natl. Acad. Sci. U. S. A.*, 112(49):E6736–43, December 2015.
27. Joanna Buchthal, Sam Weiss Evans, Jeantine Lunshof, Sam R Telford, 3rd, and Kevin M Esvelt. Mice against ticks: an experimental community-guided effort to prevent tick-borne disease by altering the shared environment. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 374(1772):20180105, May 2019.
28. Kyros Kyrrou, Andrew M Hammond, Roberto Galizi, Nace Kranjc, Austin Burt, Andrea K Beaghton, Tony Nolan, and Andrea Crisanti. A CRISPR-Cas9 gene drive targeting doublesex causes complete population suppression in caged anopheles gambiae mosquitoes. *Nat. Biotechnol.*, 36(11):1062–1066, December 2018.
29. Mohammad KaramiNejadRanjbar, Kolja N Eckermann, Hassan M M Ahmed, Héctor M Sánchez C, Stefan Dippel, John M Marshall, and Ernst A Wimmer. Consequences of resistance evolution in a cas9-based sex conversion-suppression gene drive for insect pest management. *Proc. Natl. Acad. Sci. U. S. A.*, 115(24):6189–6194, June 2018.
30. Philip J Lester, Mariana Bulgarella, James W Baty, Peter K Dearden, Joseph Guhlin, and John M Keane. The potential for a CRISPR gene drive to eradicate or suppress globally invasive social wasps. *Sci. Rep.*, 10(1):12398, July 2020.
31. Andrew Hammond, Roberto Galizi, Kyros Kyrrou, Alekos Simoni, Carla Siniscalchi, Dimitris Katsanos, Matthew Gribble, Dean Baker, Eric Marois, Steven Russell, et al. A crisp-cas9 gene drive system targeting female reproduction in the malaria mosquito vector anopheles gambiae. *Nature biotechnology*, 34(1):78–83, 2016.
32. Nikolay P Kandul, Junru Liu, Jared B Bennett, John M Marshall, and Omar Akbari. A home and rescue gene drive efficiently spreads and persists in populations. *bioRxiv*, 2020.
33. Gerard Terradas, Anna B Buchman, Jared B Bennett, Isaiiah Shriver, John M Marshall, Omar S Akbari, and Ethan Bier. Inherently confinable split-drive systems in drosophila. *Nature communications*, 12(1):1–12, 2021.
34. Nicky R Faber, Gus R McFarlane, R Chris Gaynor, Ivan Pocrnic, C Bruce A Whitelaw, and Gregor Gorjanc. Novel combination of crisp-based gene drives eliminates resistance and localises spread. *Scientific reports*, 11(1):1–15, 2021.
35. Noble I Egekwu, Francisco Posada, Daniel E Sonenshine, and Steven Cook. Using an in vitro system for maintaining varroa destructor mites on apis mellifera pupae as hosts: studies of mite longevity and feeding behavior. *Exp. Appl. Acarol.*, 74(3):301–315, March 2018.
36. Cameron J Jack, Ping-Li Dai, Edzard van Santen, and James D Ellis. Comparing four methods of rearing varroa destructor in vitro. *Exp. Appl. Acarol.*, 80(4):463–476, April 2020.
37. Maeva A Techer, Rahul V Rane, Miguel L Grau, John M K Roberts, Shawn T Sullivan, Ivan Liachko, Anna K Childers, Jay D Evans, and Alexander S Mikheyev. Divergent evolutionary trajectories following speciation in two ectoparasitic honey bee mites. *Communications Biology*, 2(1):357, October 2019.
38. Zachary Y Huang, Guowu Bian, Zhiyong Xi, and Xianbing Xie. Genes important for survival or reproduction in varroa destructor identified by RNAi. *Insect Sci.*, 26(1):68–75, February 2019.
39. Wannes Dermauw, Wim Jonckheere, Maria Riga, Ioannis Livadaras, John Vontas, and Thomas Van Leeuwen. Targeted mutagenesis using CRISPR-Cas9 in the chelicerate herbivore tetranychus urticae. *Insect Biochem. Mol. Biol.*, 120:103347, May 2020.
40. Anthony A James. Gene drive systems in mosquitoes: rules of the road. *Trends Parasitol.*, 21(2):64–67, February 2005.
41. Steven P Sinkins and Fred Gould. Gene drive systems for insect disease vectors. *Nat. Rev. Genet.*, 7(6):427–435, June 2006.
42. Thomas A A Prowse, Phillip Cassey, Joshua V Ross, Chandran Pfitzner, Talia A Wittmann, and Paul Thomas. Dodging silver bullets: good CRISPR gene-drive design is critical for eradicating exotic vertebrates. *Proc. Biol. Sci.*, 284(1860), August 2017.
43. Robert L Unckless, Andrew G Clark, and Philipp W Messer. Evolution of resistance against crisp/cas9 gene drive. *Genetics*, 205(2):827–841, 2017.
44. Charleston Noble, Ben Adiam, George M Church, Kevin M Esvelt, and Martin A Nowak. Current CRISPR gene drive systems are likely to be highly invasive in wild populations. *Elife*, 7, June 2018.
45. James J Bull. Lethal gene drive selects inbreeding. *Evol Med Public Health*, 2017(1):1–16, December 2016.
46. Jun Li, Ofer Aidlin Harari, Anna-louise Doss, Linda L Walling, Peter W Atkinson, Shai Morin, and Bruce E Tabashnik. Can CRISPR gene drive work in pest and beneficial haplodiploid species? *Evol. Appl.*, 9:1759, June 2020.
47. Ingemar Fries, Scott Camazine, and James Sneyd. Population dynamics of varroa jacobsoni: A model and a review. *null*, 75(1):5–28, January 1994.
48. Johan N M Calis, Ingemar Fries, and Stephen C Rylie. Population modelling of varroa jacobsoni oud. *Apidologie*, 30(2-3):111–124, 1999.
49. Stephen Martin. A population model for the ectoparasitic mite varroa jacobsoni in honey bee (apis mellifera) colonies. *Ecol. Modell.*, 109(3):267–281, June 1998.
50. Lilia I De Guzman, Thomas E Rinderer, and Amanda M Frake. Growth of varroa destructor (acar: Varroidae) populations in russian honey bee (hymenoptera: Apidae) colonies. *Annals of the Entomological Society of America*, 100(2):187–195, 2007.
51. Alexis L Beaurepaire, Klemens J Krieger, and Robin F A Moritz. Seasonal cycle of inbreeding and recombination of the parasitic mite varroa destructor in honeybee colonies and its implications for the selection of acaricide resistance. *Infect. Genet. Evol.*, 50:49–54, June 2017.
52. Tom J de Jong. Gene drives do not always increase in frequency: from genetic models to risk assessment. *Journal of Consumer Protection and Food Safety*, 12(4):299–307, 2017.
53. P; Currie Gatien. Timing of acaricide treatments for control of low-level populations of varroa destructor (acar: Varroidae) and implications for colony performance of honey bees. *Ottawa Law Rev.*, 135(5):749–763, October 2003.
54. Willem J Boot, David JA Sisselaar, Johan NM Calis, and Joop Beetsma. Factors affecting invasion of varroa jacobsoni (acar: Varroidae) into honeybee, apis mellifera (hymenoptera: Apidae), brood cells. *Bulletin of Entomological Research*, 84(1):3–10, 1994.
55. Noble I Egekwu, Francisco Posada, Daniel E Sonenshine, and Steven Cook. Using an

- in vitro system for maintaining varroa destructor mites on apis mellifera pupae as hosts: studies of mite longevity and feeding behavior. *Exp. Appl. Acarol.*, 74(3):301–315, 2018.
56. Nonno Hasegawa, Maeva Techer, and Alexander S Mikheyev. A toolkit for studying varroa genomics and transcriptomics: preservation, extraction, and sequencing library preparation. *BMC Genomics*, 22(1):54, January 2021.
 57. Luis Medina Medina, Stephen J Martin, Laura Espinosa-Montaña, and Francis L W Ratnieks. Reproduction of varroa destructor in worker brood of africanized honey bees (apis mellifera). *Exp. Appl. Acarol.*, 27(1-2):79–88, 2002.
 58. Gloria DeGrandi-Hoffman and Robert Curry. A mathematical model of varroa mite (varroa destructor anderson and truemana) and honeybee (apis mellifera L.) population dynamics. *Int. J. Acarology*, 30(3):259–274, September 2004.
 59. MD Ifantidis. Parameters of the population dynamics of the varroa mite on honeybees. *Journal of Apicultural Research*, 23(4):227–233, 1984.
 60. L E Brettell and S J Martin. Oldest varroa tolerant honey bee population provides insight into the origins of the global decline of honey bees. *Sci. Rep.*, 7:45953, April 2017.
 61. Sandra Barroso-Arévalo, Eduardo Fernández-Carrión, Joaquín Goyache, Fernando Molero, Francisco Puerta, and José Manuel Sánchez-Vizcaino. High load of deformed wing virus and varroa destructor infestation are related to weakness of honey bee colonies in southern Spain. *Front. Microbiol.*, 10:1331, June 2019.
 62. Gennaro Di Prisco, Desiderato Annoscia, Marina Margiotta, Rosalba Ferrara, Paola Varrichio, Virginia Zanni, Emilio Caprio, Francesco Nazzi, and Francesco Pennacchio. A mutualistic symbiosis between a parasitic mite and a pathogenic virus undermines honey bee immunity and health. *Proc. Natl. Acad. Sci. U. S. A.*, 113(12):3203–3208, March 2016.
 63. R W Currie and P Gatién. Timing acaricide treatments to prevent varroa destructor (acari: Varroidae) from causing economic damage to honey bee colonies. *Canadian Entomologist: Ottawa*, 138(2):238–252, April 2006.
 64. Keith S Delaplane and W Michael Hood. Economic threshold for varroa jacobsoni oud. in the southeastern USA. *Apidologie*, 30(5):383–395, 1999.
 65. R M Goodwin, M A Taylor, H M McBrydie, and H M Cox. Drift of varroa destructor-infested worker honey bees to neighbouring colonies. *J. Apic. Res.*, 45(3):155–156, January 2006.
 66. David T Peck, Michael L Smith, and Thomas D Seeley. Varroa destructor mites can nimbly climb from flowers onto foraging honey bees. *PLoS One*, 11(12):e0167798, December 2016.
 67. David Thomas Peck and Thomas Dyer Seeley. Mite bombs or robber lures? the roles of drifting and robbing in varroa destructor transmission from collapsing honey bee colonies to their neighbors. *PLoS One*, 14(6):e0218392, June 2019.
 68. Thomas D Seeley and Michael L Smith. Crowding honeybee colonies in apiaries can increase their vulnerability to the deadly ectoparasite varroa destructor. *Apidologie*, 46(6):716–727, November 2015.
 69. Sean P Leonard, J Elijah Powell, Jiri Perutka, Peng Geng, Luke C Heckmann, Richard D Horak, Bryan W Davies, Andrew D Ellington, Jeffrey E Barrick, and Nancy A Moran. Engineered symbionts activate honey bee immunity and limit pathogens. *Science*, 367(6477):573–576, 2020.
 70. R Core Team et al. R: A language and environment for statistical computing, 2013.
 71. R Chris Gaynor, Gregor Gorjanc, and John M Hickey. Alphasimr: An r-package for breeding program simulations. *BioRxiv*, 2020.
 72. M Delia Allen. The effect of a plentiful supply of drone comb on colonies of honeybees. *Journal of apicultural research*, 4(2):109–119, 1965.
 73. R Büchler, A Uzunov, M Kovačić, J Prešern, M Pietropaoli, F Hatjina, B Pavlov, L Charistos, G Formato, E Galarza, et al. Summer brood interruption as integrated management strategy for effective varroa control in Europe. *Journal of Apicultural Research*, pages 1–10, 2020.
 74. S J Martin and D Kemp. Average number of reproductive cycles performed by varroa jacobsoni in honey bee (apis mellifera) colonies. *Journal of Apicultural research*, 36(3-4):113–123, 1997.
 75. Ingemar Fries and Peter Rosenkranz. Number of reproductive cycles of varroa jacobsoni in honey-bee (apis mellifera) colonies. *Experimental & applied acarology*, 20(2):103–112, 1996.
 76. S Fuchs. Choice in varroa jacobsoni oud. between honey bee drone or workerbrood cells for reproduction. *Behavioral Ecology and Sociobiology*, 31(6):429–435, 1992.
 77. Peter Rosenkranz, Pia Aumeier, and Bettina Ziegelmann. Biology and control of varroa destructor. *Journal of invertebrate pathology*, 103:S96–S119, 2010.
 78. Francesco Nazzi and Yves Le Conte. Ecology of varroa destructor, the major ectoparasite of the western honey bee, apis mellifera. *Annu. Rev. Entomol.*, 61:417–432, 2016.
 79. S J Martin. Reproduction of varroa jacobsoni in cells of apis mellifera containing one or more mother mites and the distribution of these cells. *J. Apic. Res.*, 34(4):187–196, January 1995.

Supplementary Material

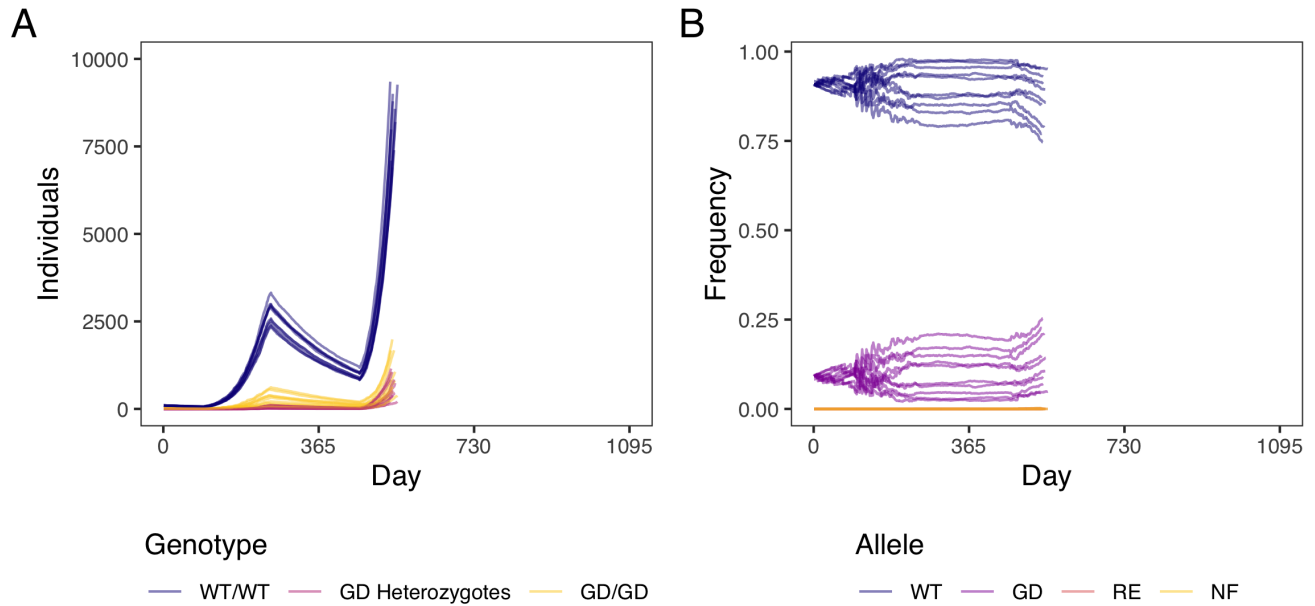


Figure S1. Model of Varroa and gene drive spread as in Figure 2C and D, but with a 10 times larger starting population: 100 wild-type Varroa instead of 10, and 10 gene drive Varroa instead of 1. The initial population size is 100 wild-type Varroa with 10 added homozygous gene drive Varroa, giving an initial gene drive frequency of 0.09. For every set of parameters, we run 10 repetitions and stop the model when the Varroa population size is over 10,000. **A)** Numbers of individuals with different genotypes. WT = wild-type and GD = gene drive. **B)** Frequencies of gene drive alleles. WT = wild-type, GD = gene drive, RE = resistant, and NF = non-functional.

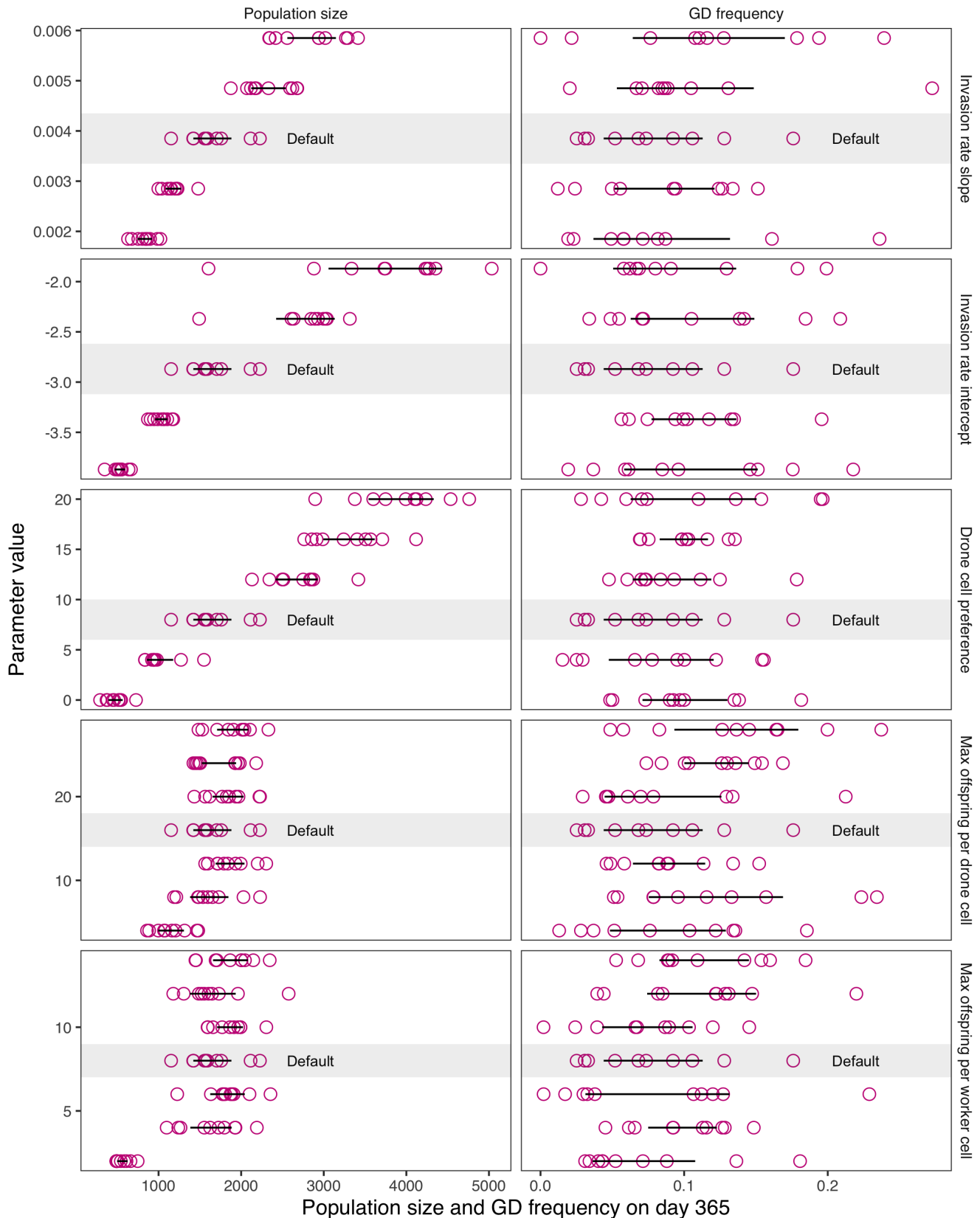
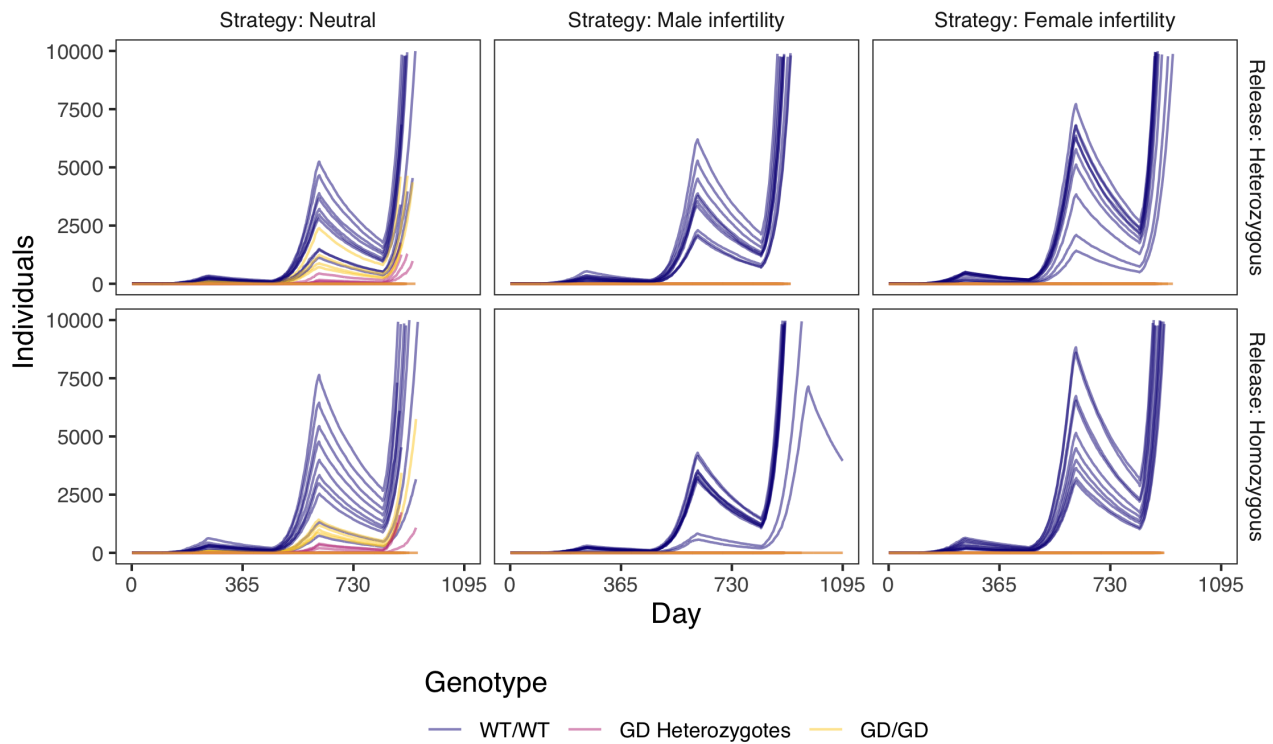


Figure S2. Sensitivity analysis of our *Varroa* model shown in Figure 2A and D. We run the model for a year with a range of parameters and on day 365, we measure both population size and gene drive (GD) frequency to see which parameter has an influence. The initial population size is 100 wild-type *Varroa* with 10 added homozygous gene drive *Varroa*, giving an initial gene drive frequency of 0.09. We vary five parameters independently: invasion rate slope (see Equation 1), invasion rate intercept (see Equation 1), drone cell preference, max offspring per drone cell (see Equation 2), and max offspring per worker cell (see Equation 2). Pink circles indicate each repetition's outcome, the black lines represent the 95% confidence interval around the mean, and the grey bar and text "Default" indicate the default parameters that are supported by literature and are used in Figure 2 and all other figures. For every set of parameters, we run 10 repetitions and stop the model when the *Varroa* population size is over 10,000.

A



B

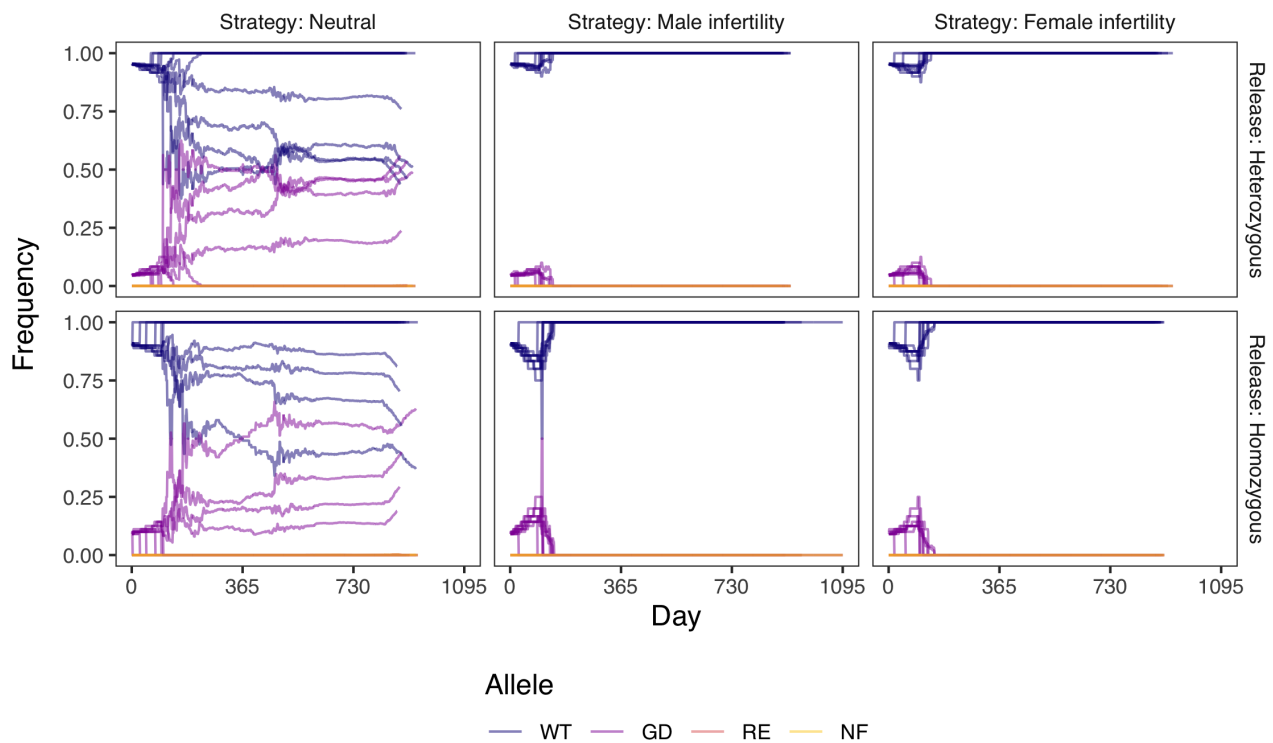


Figure S3. Model of *Varroa* and gene drive spread as in Figure 2C and D, but besides a neutral gene drive, we also model a gene drive which, when homozygous or hemizygous, causes male or female infertility. Besides the release of homozygous females as in Figure 2C and D, we also model the release of heterozygous gene drive *Varroa* females so the infertility does not immediately affect females. The initial population size is 10 wild-type *Varroa* with 1 added gene drive *Varroa*, giving an initial gene drive frequency of 0.09 for a homozygote release and 0.045 for a heterozygote release. For every set of parameters, we run 10 repetitions and stop the model when the *Varroa* population size is over 10,000. **A)** Numbers of individuals with different genotypes. WT = wild-type and GD = gene drive. **B)** Frequencies of gene drive alleles. WT = wild-type, GD = gene drive, RE = resistant, and NF = non-functional.

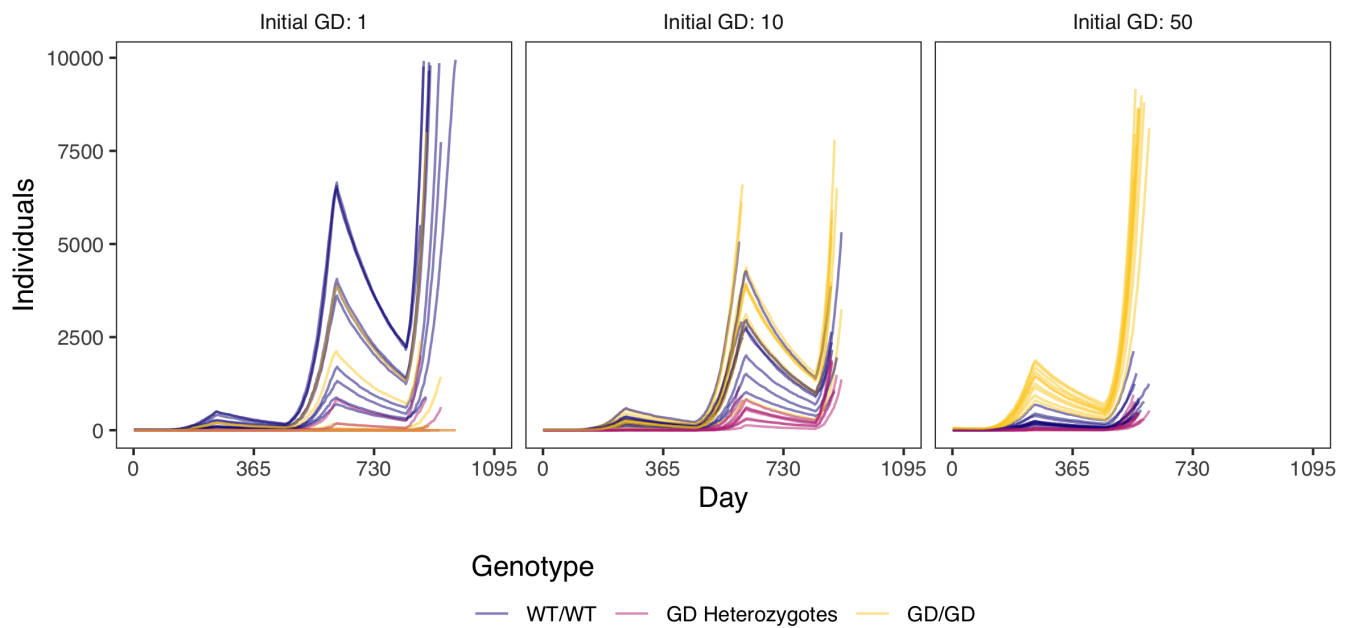


Figure S4. Numbers of individuals with three genotypes, corresponding to the allele frequencies in Figure 3 over three years with different gene drive introduction amounts. The initial population size is 10 wild-type *Varroa* with 1, 10 or 50 added homozygous gene drive *Varroa*, giving initial gene drive frequencies of 0.09, 0.50, and 0.83, respectively. WT = wild-type and GD = gene drive. For every set of parameters, we run 10 repetitions and stop the model when the *Varroa* population size is over 10,000.

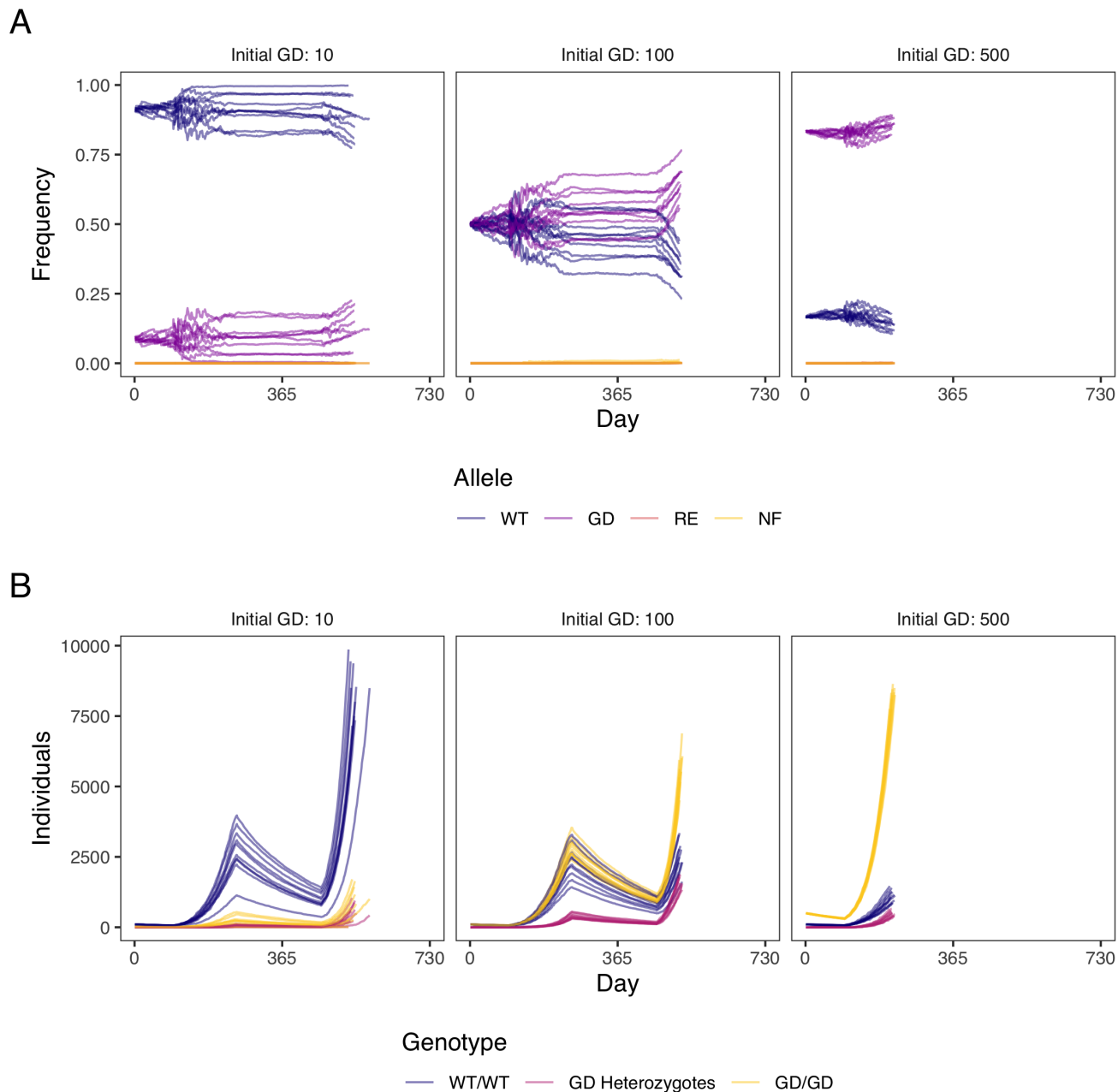


Figure S5. The same as Figure 3 and Figure S4, but with 10 times more initial Varroa. The initial population size is 100 wild-type Varroa with 10, 100 or 500 added homozygous gene drive Varroa, respectively giving initial gene drive frequencies of 0.09, 0.50, and 0.83. For every set of parameters, we run 10 repetitions and stop the model when the Varroa population size is over 10,000. **A)** Allele frequencies over three years with different gene drive introduction amounts. WT = wild-type, GD = gene drive, RE = resistant, and NF = non-functional. **B)** Numbers of individuals with three genotypes. WT = wild-type and GD = gene drive.

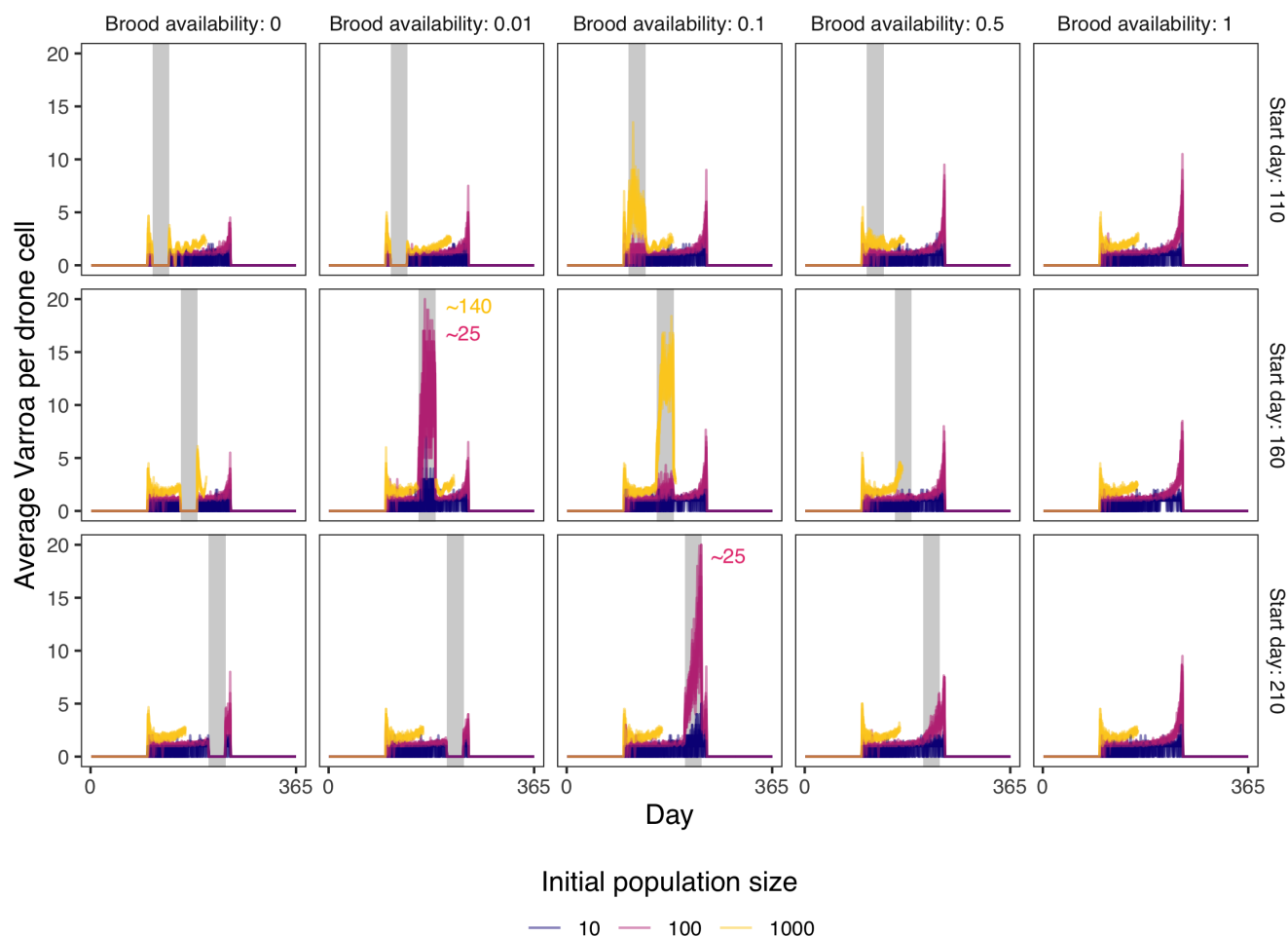


Figure S6. Average Varroa per drone cell over time for different initial population sizes, given different amounts of brood cell availability (as a fraction of the normal amount) and different brood break starting days like in Figure 4. The grey bars indicate the brood break. The “~” in two plots indicates that values were higher than 20 and thus fall off the truncated y-axis to keep the plot interpretable. The number after the “~” roughly indicates the maximum of the truncated values. The initial population sizes were 10, 100, or 1000 wild-type Varroa with the same number of gene drive Varroa on top of that, giving initial gene drive frequencies of 0.5. For every set of parameters, we run 10 repetitions and stop the model when the Varroa population size is over 10,000.

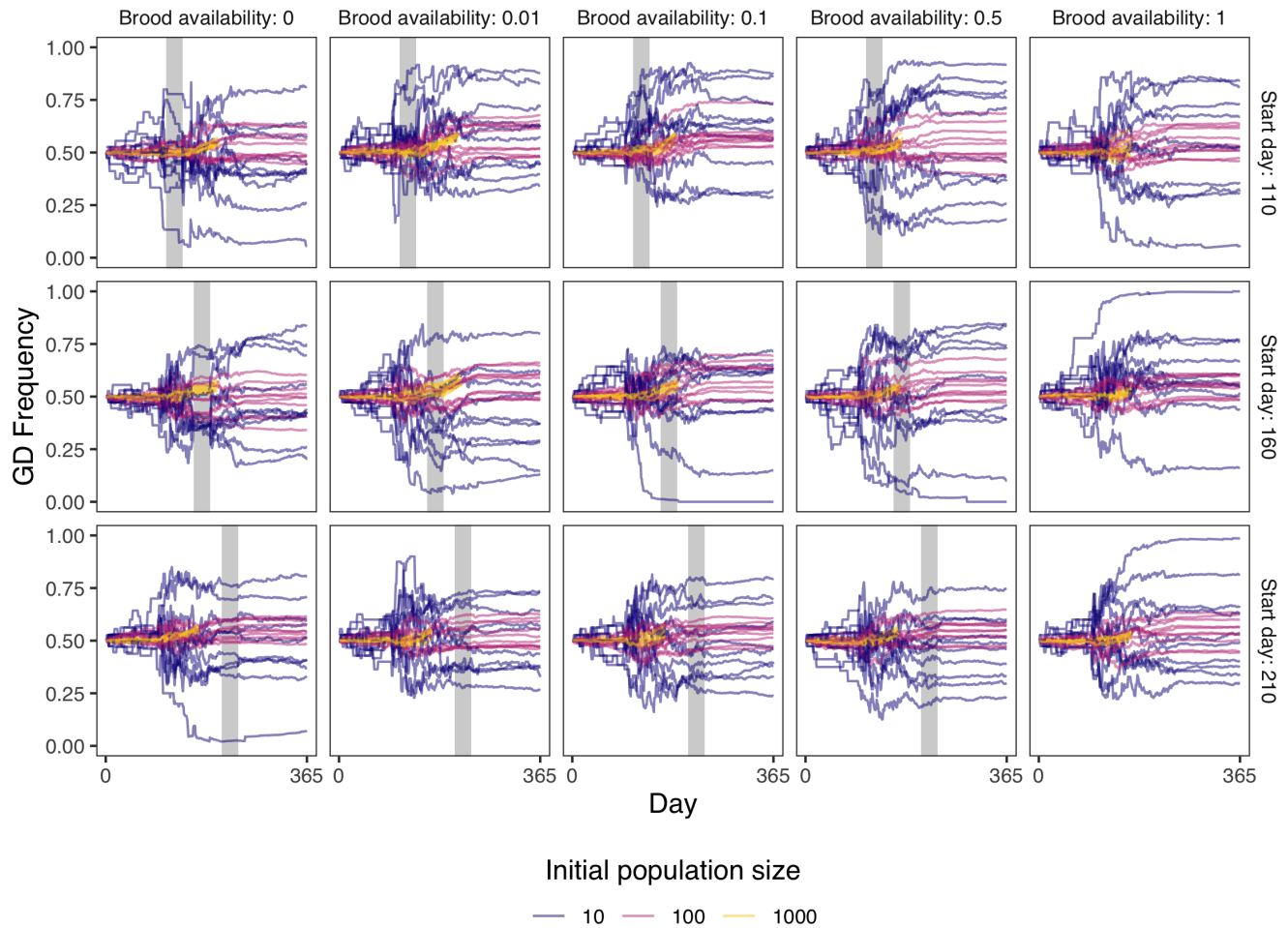


Figure S7. Gene drive (GD) allele frequency over time for different initial population sizes, given different amounts of brood cell availability (as a fraction of the normal amount) and different brood break starting days like in Figure 4. The grey bars indicate the brood break. The initial population sizes were 10, 100, or 1000 wild-type Varroa with the same number of gene drive Varroa on top of that, giving an initial gene drive frequency of 0.5. For every set of parameters, we run 10 repetitions and stop the model when the Varroa population size is over 10,000.

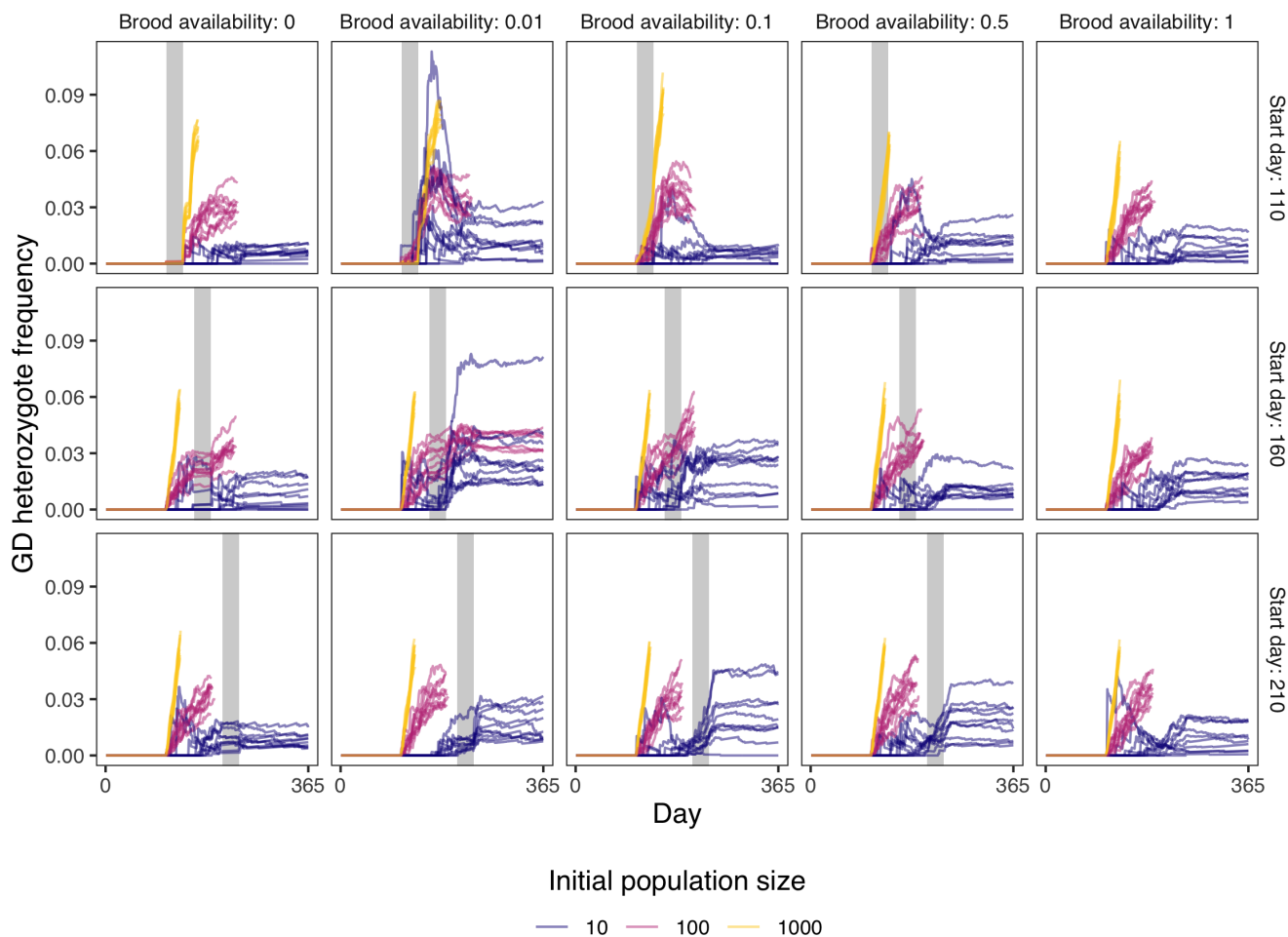


Figure S8. Gene drive (GD) heterozygote frequency over time for different initial population sizes, given different amounts of brood cell availability (as a fraction of the normal amount) and different brood break starting days like in Figure 4, but with more introduced gene drive Varroa. The initial population sizes were 10, 100, and 1000 wild-type Varroa with 100, 1000, and 5000 gene drive Varroa on top of that, respectively, giving initial gene drive frequencies of 0.91, 0.91, and 0.83. The grey bars indicate the brood break. For every set of parameters, we run 10 repetitions and stop the model when the Varroa population size is over 10,000.

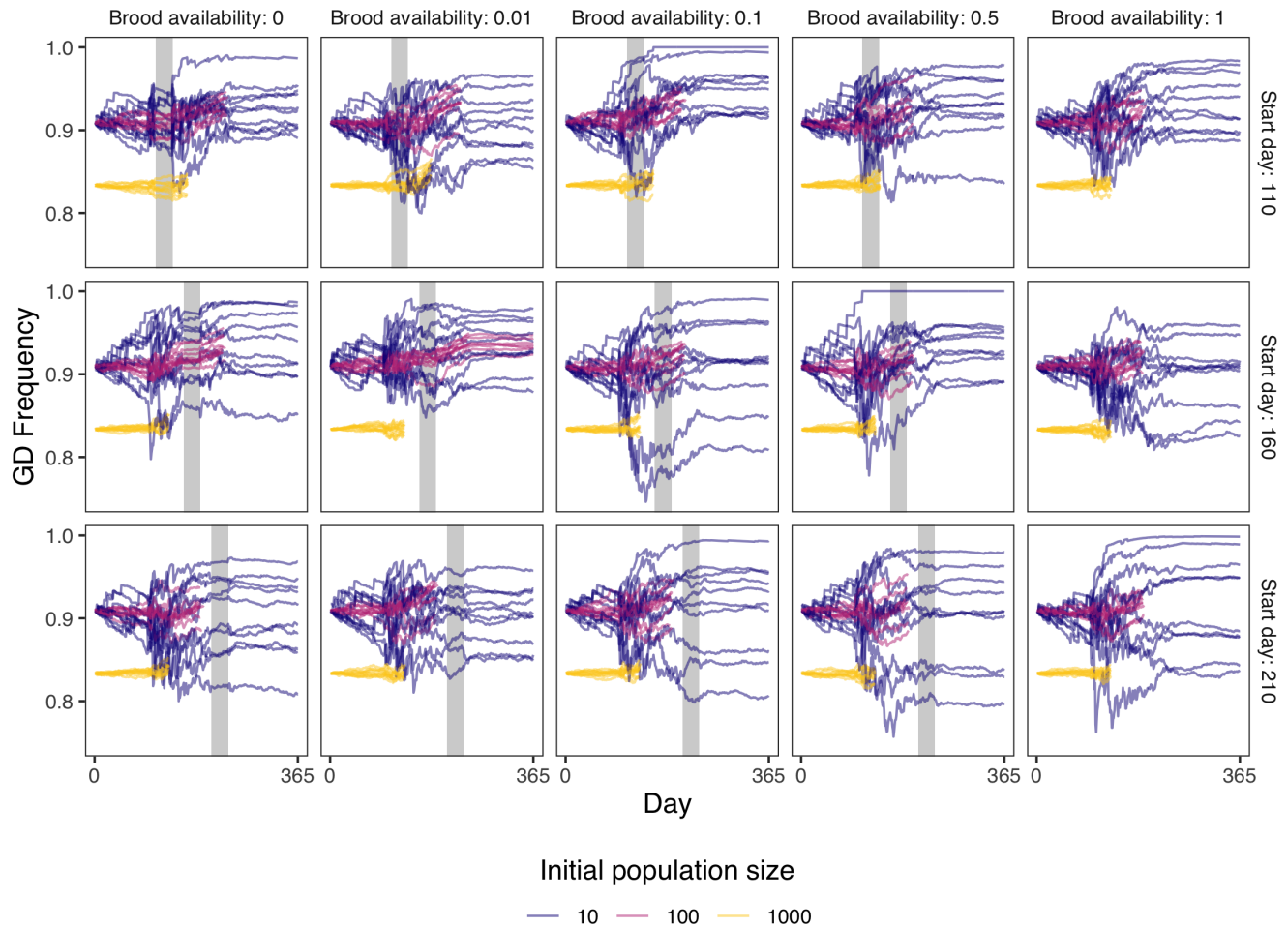


Figure S9. Gene drive (GD) allele frequency over time for different initial population sizes, given different amounts of brood cell availability (as a fraction of the normal amount) and different brood break starting days like in Figure 4, but with more introduced gene drive Varroa. The initial population sizes were 10, 100, and 1000 wild-type Varroa with 100, 1000, and 5000 gene drive Varroa on top of that, respectively, giving initial gene drive frequencies of 0.91, 0.91, and 0.83. The grey bars indicate the brood break. For every set of parameters, we run 10 repetitions and stop the model when the Varroa population size is over 10,000.

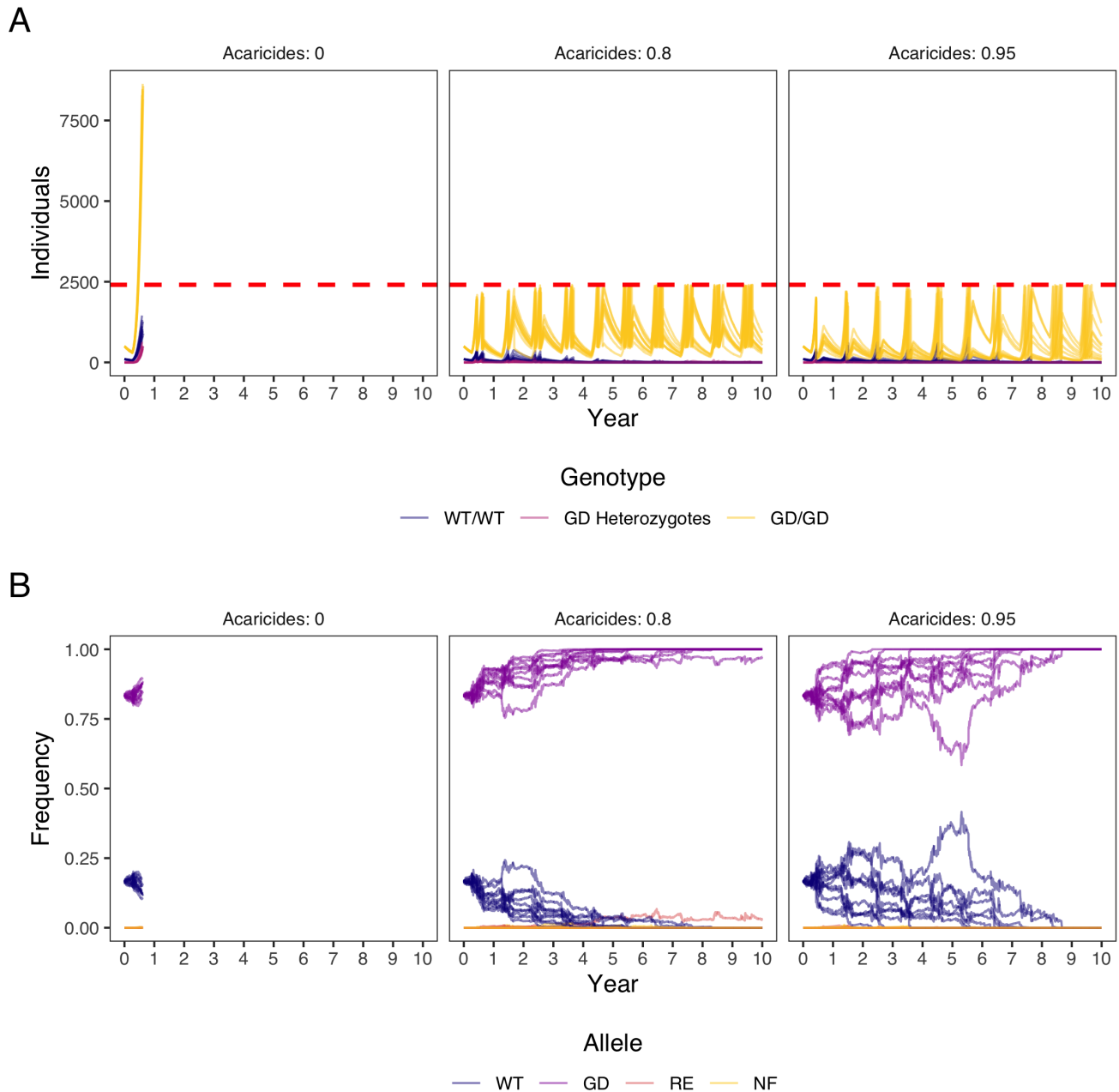


Figure S10. The spread of a gene drive while the Varroa population is suppressed with acaricides whenever the Varroa prevalence surpasses the danger threshold of 5% in summer (5 Varroa per 100 adult bees). The same as Figure 5, but with a 10 times larger starting population. The initial population size was 100 wild-type Varroa with 500 homozygous gene drive Varroa, giving an initial gene drive frequency of 0.83. For every set of parameters, we run 10 repetitions and stop the model when the Varroa population size is over 10,000. **A)** Frequencies of gene drive genotypes over time, given different intensities of acaricide treatment when the population surpasses the danger threshold. WT = wild-type, GD = gene drive. **B)** Frequencies of gene drive alleles over time, given different intensities of acaricide treatment when the population surpasses the danger threshold. WT = wild-type, GD = gene drive, RE = resistant, and NF = non-functional.

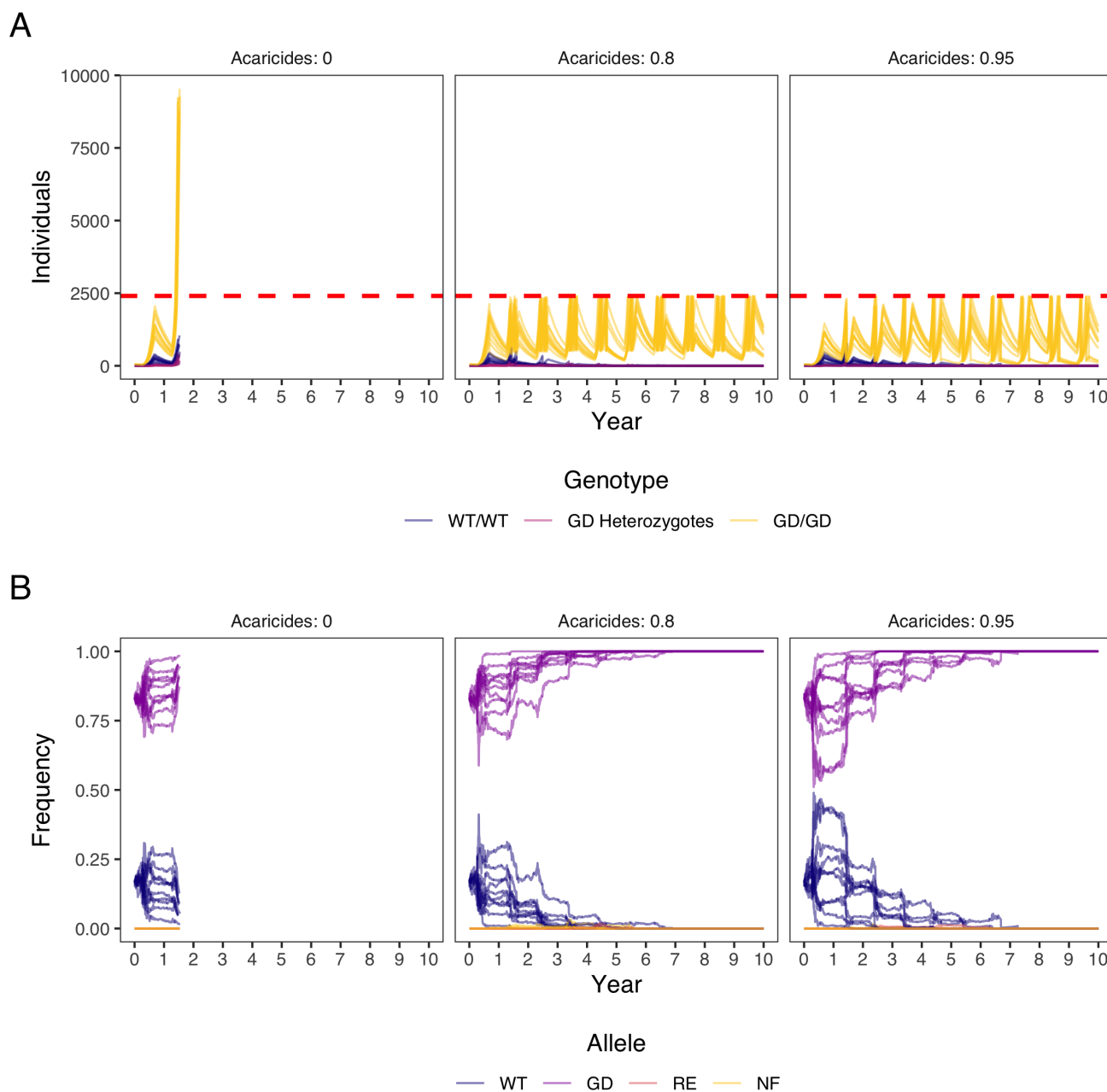


Figure S11. The spread of a gene drive while the Varroa population is suppressed with acaricides whenever the Varroa prevalence surpasses the danger threshold of 5% in summer (5 Varroa per 100 adult bees). The same as Figure 5, but now we do an extra release of 50 gene drive Varroa after every acaricide treatment. The initial population size was 10 wild-type Varroa with 50 homozygous gene drive Varroa, giving an initial gene drive frequency of 0.83. For every set of parameters, we run 10 repetitions and stop the model when the Varroa population size is over 10,000. **A)** Frequencies of gene drive genotypes over time, given different intensities of acaricide treatment when the population surpasses the danger threshold. WT = wild-type, GD = gene drive. **B)** Frequencies of gene drive alleles over time, given different intensities of acaricide treatment when the population surpasses the danger threshold. WT = wild-type, GD = gene drive, RE = resistant, and NF = non-functional.