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1 **Manipulating mosquito tolerance for arbovirus control**

2

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12

13

14 **Abstract**

15

16 The inexorable emergence of mosquito-borne arboviruses and the failure of traditional
17 vector control methods to prevent their transmission have triggered the development of
18 alternative entomological interventions to render mosquito populations incapable of
19 carrying arboviruses. Here, we use a theoretical framework to argue that decreasing
20 mosquito tolerance to arbovirus infection could be a more evolutionarily sustainable
21 disease control strategy than increasing mosquito resistance. Increasing resistance is
22 predicted to select for mutant arboviruses escaping resistance, whereas reducing
23 tolerance should lead to the death of infected vectors and thus select for mosquito-
24 attenuated arbovirus variants that are less transmissible.

25

26 **Resistance, tolerance, and the control of mosquito-borne arboviruses**

27

28 In every host-pathogen relationship, the host can survive by employing two conceptually
29 different strategies. It can resist the infection or it can limit –tolerate– the deleterious
30 effects of the infection. Distinction between these two defense strategies was made as
31 early as 1932 by Clunies-Ross in the context of gastro-intestinal parasites affecting sheep
32 farming (Clunies-Ross, 1932). He proposed that a breeding program to minimize
33 production losses due to infection would be more profitable than a breeding program to
34 increase parasite resistance *per se*. This concept was subsequently revisited in the 1980s
35 by Albers, who distinguished ‘resistance’, defined as the ability to suppress establishment
36 and/or subsequent development of infection, from ‘resilience’, defined as the ability to
37 maintain a relatively undepressed production level when infected (Albers et al., 1987).

38

39 In the 1990s, plant ecologists resurfaced the conceptual framework of a two-faceted
40 defense response relying on host resistance –the ability to limit pathogen burden– and
41 host tolerance –the ability to limit the disease severity induced by a given pathogen
42 burden (Simms and Triplett, 1994). In this literature, tolerance is usually defined as the
43 slope of host fitness against infection intensity. In the 2000s, animal parasitologists
44 borrowed this approach to measure tolerance (the reaction norm of host health to
45 pathogen burden) in a rodent malaria model (Raberg et al., 2007). This landmark study
46 demonstrated the existence of genetic variation in tolerance and a genetic trade-off
47 between resistance and tolerance in an animal host-pathogen system. Genetic variation
48 is a prerequisite for a trait to evolve in response to selective pressure. Because resistance
49 has a negative effect on pathogen fitness whereas tolerance does not, their relative
50 importance, and the extent to which their genetic basis is shared, can result in a variety
51 of evolutionary trajectories for both the host and the pathogen (Svensson and Raberg,
52 2010).

53 Following these seminal studies, a mathematical framework has been developed based
54 on empirical data from several model organisms (including the fruit fly and the mouse) to
55 distinguish between tolerance and resistance mechanisms, with the hope of improving
56 treatment and prevention of infectious disease in humans and animals (Medzhitov et al.,
57 2012; Schneider and Ayres, 2008). In this mini-review, we introduce the idea of
58 manipulating the tolerance of mosquito vectors for the control of human diseases caused
59 by mosquito-borne arboviruses, such as dengue, Zika and chikungunya (Wilder-Smith et
60 al., 2017). Hereafter, we use the term tolerance to refer to all host defense mechanisms
61 that limit ‘damage to functions and structures’ during infection, without interfering with
62 pathogen load, as was defined more than 60 years ago by plant pathologists (Caldwell et
63 al., 1958).

64

65 The failure of current methods for mosquito-borne arbovirus control has stimulated the
66 development of several innovative strategies (Achee et al., 2019). One of these
67 strategies, referred to as population modification, aims at rendering the wild vector
68 population incapable of transmitting pathogens (Flores and O'Neill, 2018). To date,
69 however, the development of novel interventions based on the population modification
70 approach has focused on increasing mosquito resistance to arbovirus infection through,
71 for example, introgression of antiviral transgenes or protective *Wolbachia* endosymbionts
72 (Kean et al., 2015). One of the main challenges to the sustainability of these strategies is
73 arbovirus evolution (Bull and Turelli, 2013). Killing a pathogen and/or reducing its growth
74 are expected to strongly select for pathogen escape mutants, which can quickly
75 undermine control. Conversely, strategies generating weaker selection for pathogen
76 counter-measures are considered more evolutionarily robust or ‘evolution-proof’ (Allen et
77 al., 2014). Here, we argue that decreasing mosquito tolerance to arbovirus infection –

78 leading to the death of infected vectors– could be a more evolutionarily sustainable
79 arbovirus control strategy than increasing mosquito resistance (Figure 1).

80
81 **What determines mosquito tolerance to arbovirus infection?**

82
83 Unlike arboviral infections in humans, which are usually acute, arboviral infections in
84 mosquitoes are persistent. Once the infection is established, the mosquito remains
85 infected for the rest of its life. Despite active replication and high viral loads, however,
86 arboviral infections in mosquitoes typically do not result in severe fitness defects.
87 Although some fitness costs of infection have been reported (e.g., (da Silveira et al., 2018;
88 Grimstad et al., 1980; Styer et al., 2007)), many other studies have failed to detect
89 experimental evidence for arbovirus virulence in mosquitoes (e.g., (Ciota et al., 2011)).
90 Overall, the cost of infection is usually modest and context-dependent (Lambrechts and
91 Scott, 2009) and mosquitoes are considered tolerant to arbovirus infections. This is
92 particularly remarkable for arboviruses such as yellow fever virus, which causes
93 substantial mortality in immunologically naïve humans but not in their mosquito vectors
94 (Barrett and Higgs, 2007). Mosquito tolerance is essential for arbovirus fitness because
95 both high viral loads and mosquito survival are necessary for successful arbovirus
96 transmission to the human host.

97
98 How do mosquitoes cope with viral infection? Response to infection does not simply
99 consist of activating immune pathways, it also encompasses a broad range of
100 physiological consequences including metabolic adaptations, stress responses, and
101 tissue repair. Critically, upon infection, the homeostatic regulation of these pathways is
102 altered. However, such alterations do not always result in increased disease severity or
103 acute infections, but can also lead to improved survival (or health) despite active

104 pathogen replication, which defines tolerance. A clear example of this conundrum can be
105 found in *Drosophila*, where the transcription factor *CrebA* is induced upon bacterial
106 infection (Troha et al., 2018). Interestingly, *CrebA*-deficient flies are more likely to die from
107 infection despite carrying the same number of bacteria as wildtype flies. *CrebA* is
108 expressed in the fat body, an organ analogous to the mammalian liver and adipose
109 tissues (Sondergaard, 1993), where it regulates the transcription of multiple secretory
110 pathway genes. Loss of *CrebA* during infection triggers endoplasmic reticulum stress,
111 which is sufficient to sensitize flies to infection. These results suggest that immune
112 tolerance modulates host physiology to prevent the deleterious effect of infection-
113 associated cellular stress.

114

115 There is increasing evidence to suggest that viral infections in insects cause intracellular
116 stress and that the cellular management of stress favors virus replication. For example,
117 dengue virus replication in mosquito cells is promoted by activation of the PERK pathway
118 during endoplasmic reticulum stress (Hou et al., 2017), autophagy (Brackney, 2017) and
119 oxidative stress (Chen et al., 2017; Chen et al., 2012; Chen et al., 2011; Riahi et al.,
120 2019). Cell homeostasis in regulating infection can thus be tuned by both the virus and
121 the immune system.

122

123 Pathogens often manipulate the behavior of their host in order to enhance their own
124 transmission (Hughes and Libersat, 2019). Such behavioral manipulations have been
125 widely observed in mosquito vectors of human pathogens (Hurd, 2003; Lefevre and
126 Thomas, 2008). Behavioral changes of infected vectors are often deviations in the
127 degree and timing of normal behaviors, including host seeking, host-attack persistence
128 and blood-feeding efficiency (Murdock et al., 2017). For example, *Aedes triseriatus*
129 females infected by LaCrosse virus tend to probe more, engorge less and reef more

130 often than non-infected siblings (Grimstad et al., 1980; Jackson et al., 2012). In some
131 cases, however, the arbovirus-induced behavioral changes in mosquitoes seem to
132 reduce virus transmission potential. For example, West Nile virus infection reduced
133 host-seeking activity of *Culex pipiens* females and did not induce a shift in their host
134 preference toward birds (Vogels et al., 2017). In those cases, behavioral changes are
135 likely side effects resulting from immune responses and infection-related alterations in
136 host physiology (Murdock et al., 2017).

137

138 To the best of our knowledge, no study to date has examined the possible manipulation
139 of mosquito tolerance mechanisms by arboviruses. It would seem advantageous for an
140 arbovirus to promote mosquito tolerance to infection, which would allow high viral loads
141 (and therefore high transmission rate) without compromising mosquito survival (and
142 therefore extending transmission duration). An important first step to address this
143 question is to elucidate the molecular mechanisms of tolerance.

144

145 Recent studies to decipher tolerance mechanisms in animals encompass various
146 experimental models, including *Drosophila* infection by bacteria (Ayres et al., 2008) or
147 RNA viruses (Merkling et al., 2015; Teixeira et al., 2008), mouse infection by
148 *Trypanosoma brucei* (Olivera et al., 2016), *Plasmodium chabaudi* (Raberg et al., 2007)
149 or influenza virus (Furuya et al., 2015), and monarch butterfly infection by protozoan
150 parasites (Altizer et al., 2015). Although significant progress has been made, the genes
151 and processes that control tolerance remains elusive. Besides canonical immune
152 pathways, various molecular pathways that are activated upon infection could contribute
153 to tolerance to infection.

154

155 **Can we manipulate mosquito tolerance to reduce arbovirus transmission?**

156

157 In a recent study, we showed that mosquito tolerance to arbovirus infection depends on
158 cellular reverse transcriptase (RT) activity (Goic et al., 2016). Fragments of RNA viruses
159 such as dengue and chikungunya viruses are reverse transcribed by endogenous RT
160 activity and the resulting viral DNA is necessary for mosquito tolerance. Preventing viral
161 DNA synthesis with the RT inhibitor azidothymidine (AZT) results in reduced mosquito
162 survival without significant changes in viral loads (Figure 2). Evidence of mosquito death
163 following arbovirus infection in the absence of viral DNA provides the proof of principle
164 that infected mosquitoes can be selectively eliminated through the loss of tolerance.

165

166 The rationale behind reducing mosquito tolerance rather than increasing resistance to
167 arbovirus infection is that loss of tolerance is expected to be more evolutionarily
168 sustainable. According to evolutionary theory, reducing tolerance should select for
169 arbovirus variants that are less virulent to their vector (i.e., attenuated). This is in contrast
170 with increasing resistance, which is predicted to select for arbovirus variants that are more
171 virulent to their vectors. When mosquito tolerance is reduced, arbovirus attenuation
172 results from a lower evolutionary optimum for viral load, which is also predicted to result
173 in lower transmissibility due to the expected positive relationship between viral load and
174 transmission rate (Figure 3).

175

176 The evolutionary response to an intervention that targets mosquito-arbovirus interactions
177 can be examined from both the mosquito and the arbovirus perspectives. There are two
178 reasons for which the evolutionary response of mosquitoes is unlikely to undermine an
179 intervention due to the arbovirus. First, as mentioned above, the deleterious effects of
180 arbovirus infection are generally modest and the mosquito fitness is largely maintained.
181 Second, the prevalence of arbovirus infection in natural mosquito populations is

182 exceedingly low. For example, dengue virus was only detected in 0.1% of *Aedes aegypti*
183 mosquitoes randomly collected in a highly endemic region of Thailand (Yoon et al., 2012).
184 The prevalence reached about 1% when mosquitoes were collected in households with
185 evidence of recent dengue virus transmission (Yoon et al., 2012). In light of this, it is
186 reasonable to suggest that arboviruses do not represent a major selective pressure
187 driving the evolution of mosquito populations in natural settings. Thus, manipulation of
188 mosquito resistance and tolerance to arbovirus infection should not lead to significant
189 selection or counter-selection due to the arbovirus selective pressure. For instance, it
190 means that the death of arbovirus-infected mosquitoes following the loss of tolerance is
191 unlikely to drive the evolution of mosquitoes to restore higher tolerance.

192

193 The evolutionary response that deserves more attention is that of the arbovirus. Because
194 transmission by mosquitoes is an essential step of the viral lifecycle, any intervention that
195 modifies mosquito-arbovirus interactions to reduce transmission may select for arbovirus
196 mutants that escape or counteract the mechanism of the intervention as a reduction of
197 transmission directly translates into a decrease in arbovirus fitness. In the case of
198 increased mosquito resistance by genetic engineering or *Wolbachia* transfection (Kean
199 et al., 2015), reducing viral load will directly result in a reduction of transmission rate.
200 Natural selection will thus favor arbovirus mutants that can restore a higher transmission
201 rate by evading or suppressing the resistance mechanism. It is worth noting that recent
202 developments in genetic engineering strategies, such as simultaneously targeting
203 multiple conserved regions of the viral genome, or different steps of the viral cycle, will
204 minimize the probability of viral escape (Buchman et al., 2019). In the case of decreased
205 mosquito tolerance, the transmission rate will remain unchanged because, by definition,
206 the viral load is unchanged. However, the duration of transmission will decrease because
207 infected vectors have a shortened lifespan. In that case, natural selection will favor

208 arbovirus mutants that are less virulent (attenuated) in the vector to restore a longer
209 duration of transmission. The extent to which the viral load will decrease depends on the
210 new evolutionary optimum determined by the trade-off between virulence and
211 transmission rate (Figure 3). We cannot ignore the possibility that arbovirus mutants with
212 reduced virulence in the mosquito may have increased virulence in the vertebrate host.
213 Future studies will be necessary to evaluate this possibility and determine whether
214 reduced transmissibility could be offset by increased virulence in the vertebrate host.

215

216 Note that our reasoning to compare the evolutionary response to tolerance-based and
217 resistance-based strategies for disease control relies on at least three simplifying
218 assumptions that condition our conclusions. First, we assume that manipulation of
219 mosquito resistance and tolerance will not result in major fitness costs in the absence of
220 the arbovirus. However, genetic manipulation of mosquitoes may result in constitutive
221 fitness costs due to inbreeding and/or transgene expression (Moreira et al., 2004).
222 Second, we assume that the change in mosquito resistance or tolerance is specific to
223 arboviral infections and does not influence other viral infections of mosquitoes, such as
224 infection by insect-specific viruses. *Wolbachia* transfection in *Ae. aegypti*, for example,
225 suppresses replication of some insect-specific viruses (Schnettler et al., 2016), and
226 possibly enhances that of others (Amuzu et al., 2018). It is likely that natural tolerance
227 mechanisms act across a broad spectrum of viruses. Therefore, specificity against
228 human-pathogenic arboviruses could be achieved by complementing a general but subtle
229 loss of tolerance with a more specific, artificial suicide-mediating mechanism. Third, we
230 assume that we can manipulate resistance and tolerance independently from each other.
231 Resistance and tolerance were found to be genetically correlated in a mouse model
232 (Raberg et al., 2007), reflecting the existence of pleiotropic genes that contribute to both
233 phenotypes. The endoribonuclease Dicer-2, for example is a cornerstone of mosquito

234 resistance to arbovirus infection (Sanchez-Vargas et al., 2009) but it may also contribute
235 to tolerance through the viral DNA biogenesis (Poirier et al., 2018).

236

237 **Concluding remarks**

238

239 The proof of principle that we can manipulate mosquito tolerance (Goic et al., 2016)
240 supports the idea that reducing mosquito tolerance to arbovirus infection could be used
241 as a disease control strategy (Figure 1). In this review, we have provided theoretical
242 arguments supporting the idea that reducing tolerance should be more evolutionarily
243 robust than increasing mosquito resistance to arbovirus infection. However, a number of
244 scientific, logistical and ethical challenges remain to be evaluated before converting this
245 idea into a realistic, and sustainable intervention for disease control. Additional
246 knowledge is necessary to better understand the mechanisms of mosquito tolerance and
247 their specificity. The strategy relies on the assumption that off-target effects are minimal
248 and therefore loss of tolerance is an ecologically sound strategy. In other words, the loss
249 of tolerance must be achieved specifically for arbovirus-infected mosquitoes, in the
250 absence of fitness cost for arbovirus-free mosquitoes. Understanding the mechanisms of
251 tolerance will be essential to design a method to safely and specifically manipulate it.
252 Moreover, the mechanism employed to reduce mosquito tolerance will have to be
253 delivered and remain effective under field conditions. Delivery may rely on mass releases
254 of gene drive systems that are being developed for other candidate methods of vector
255 population modification (Flores and O'Neill, 2018). Like for other innovative vector control
256 strategies under development, the proof of concept is only the very first step towards
257 successful implementation (Achee et al., 2019). The public health value of an
258 entomological intervention is ultimately established through large-scale field trials with
259 epidemiological endpoints. Such a difficult and time-consuming process makes it crucial

260 to preferentially invest in candidate disease control strategies that are evolutionarily
261 sustainable.

262

263

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265

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413

414 **Figures Legends**

415

416

417 **Figure 1. Reducing mosquito tolerance is more evolutionarily sustainable than**
418 **increasing resistance to arbovirus infection.** This simple conceptual framework
419 describes the expected short-term and long-term consequences of manipulating
420 resistance and tolerance to arbovirus infection in mosquitoes.

421

422 **Figure 2. Inhibition of reverse transcriptase activity results in the loss of mosquito**
423 **tolerance to chikungunya virus infection.**

424 *Aedes albopictus* mosquitoes treated daily with 10 mg/ml of reverse transcriptase inhibitor
425 azidothymidine (AZT) and infected with chikungunya virus (CHIKV) die faster than
426 controls exposed to AZT alone or infected with CHIKV alone (**A**). Despite the accelerated
427 death of AZT-treated mosquitoes upon CHIKV infection, viral loads over time are similar
428 to that of untreated controls (**B**). Data from Goic *et al.* (Goic et al., 2016).

429

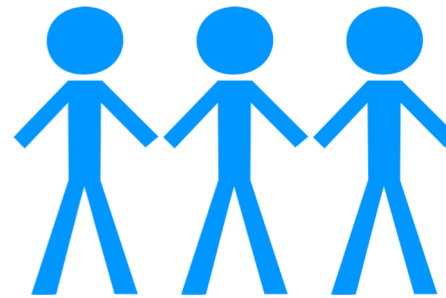
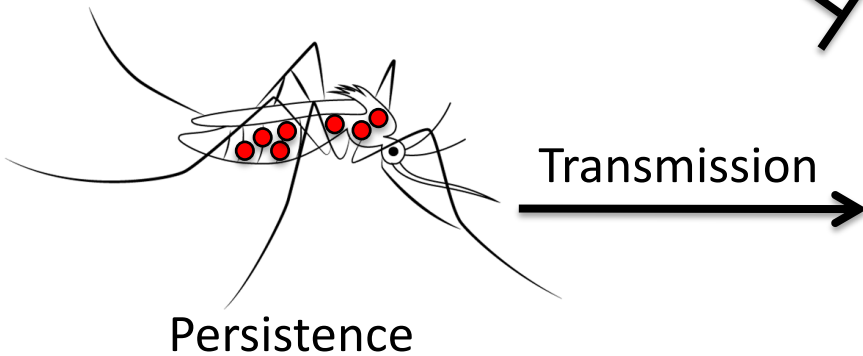
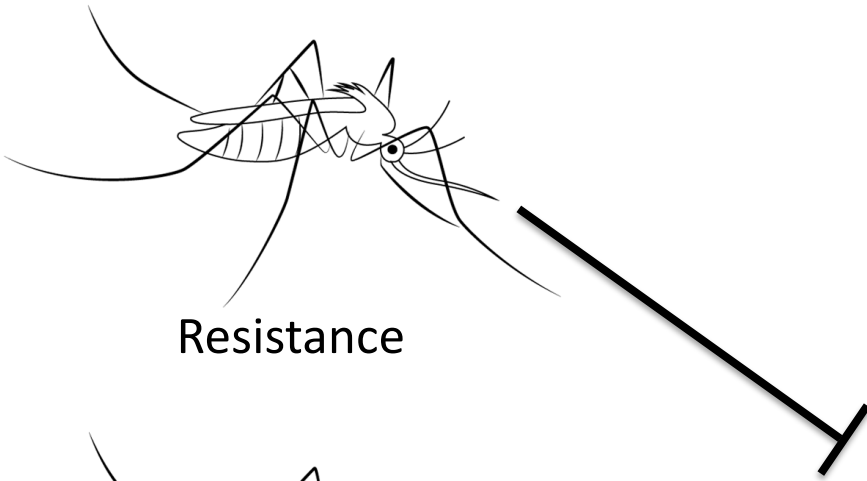
430 **Figure 3. Decreasing vector tolerance is expected to drive the evolution of virus**
431 **attenuation in the vector and reduced transmissibility.**

432 Virus fitness is a function of the duration of transmission (the vector lifespan, here denoted
433 α) and the amount of transmission per unit of time (transmission rate, here denoted β) .
434 A trade-off arises for the virus because the transmission rate β is positively correlated
435 with viral load (**A**), whereas the transmission duration α is negatively correlated with viral
436 load (**B**). This trade-off results in a virus fitness optimum at an intermediate value of viral
437 load (**C**). When vector tolerance is reduced, the relationship between vector lifespan and
438 viral load has a steeper slope (**B**) and it translates into a lower viral load optimum (**C**) and
439 a reduced transmission rate (**A**).

Consequences of increasing resistance

Short term: stop transmission

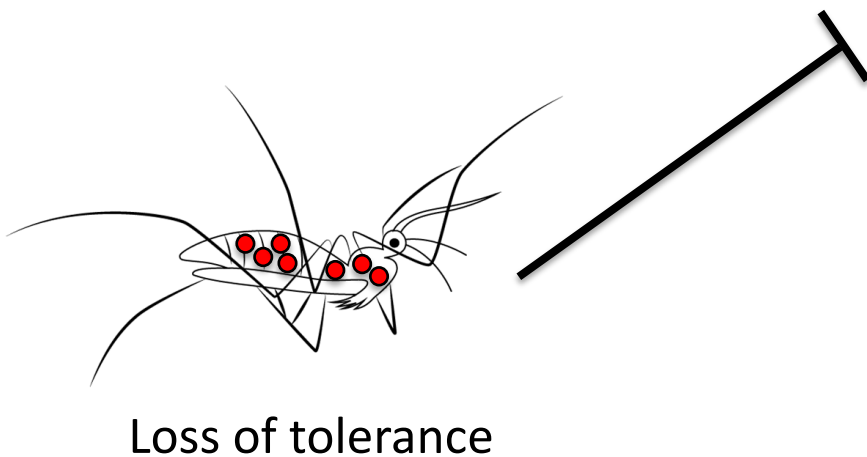
Long term: evolutionary arms race between virus and resistance mechanism

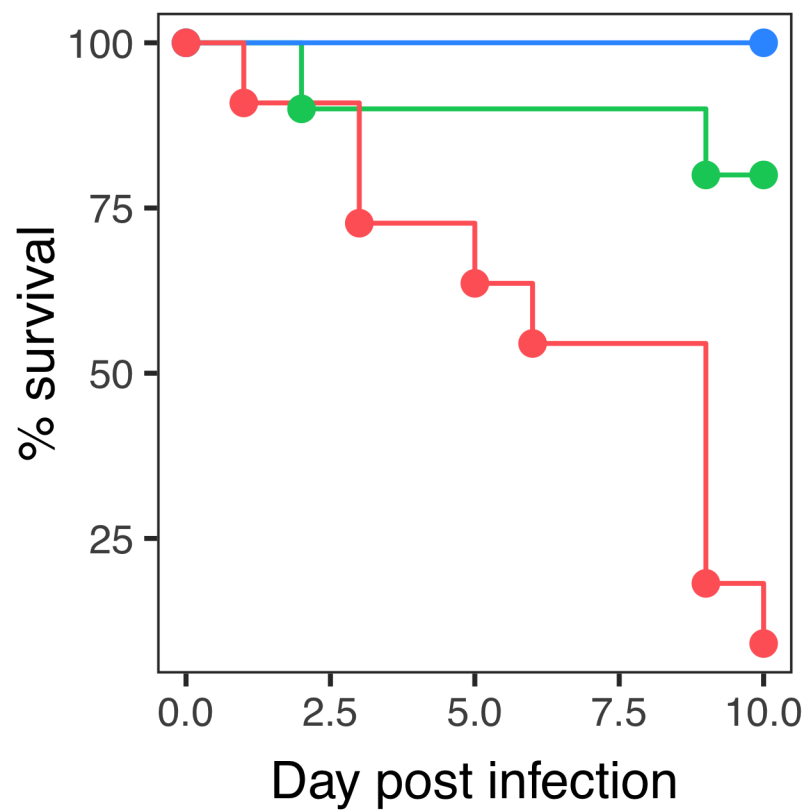
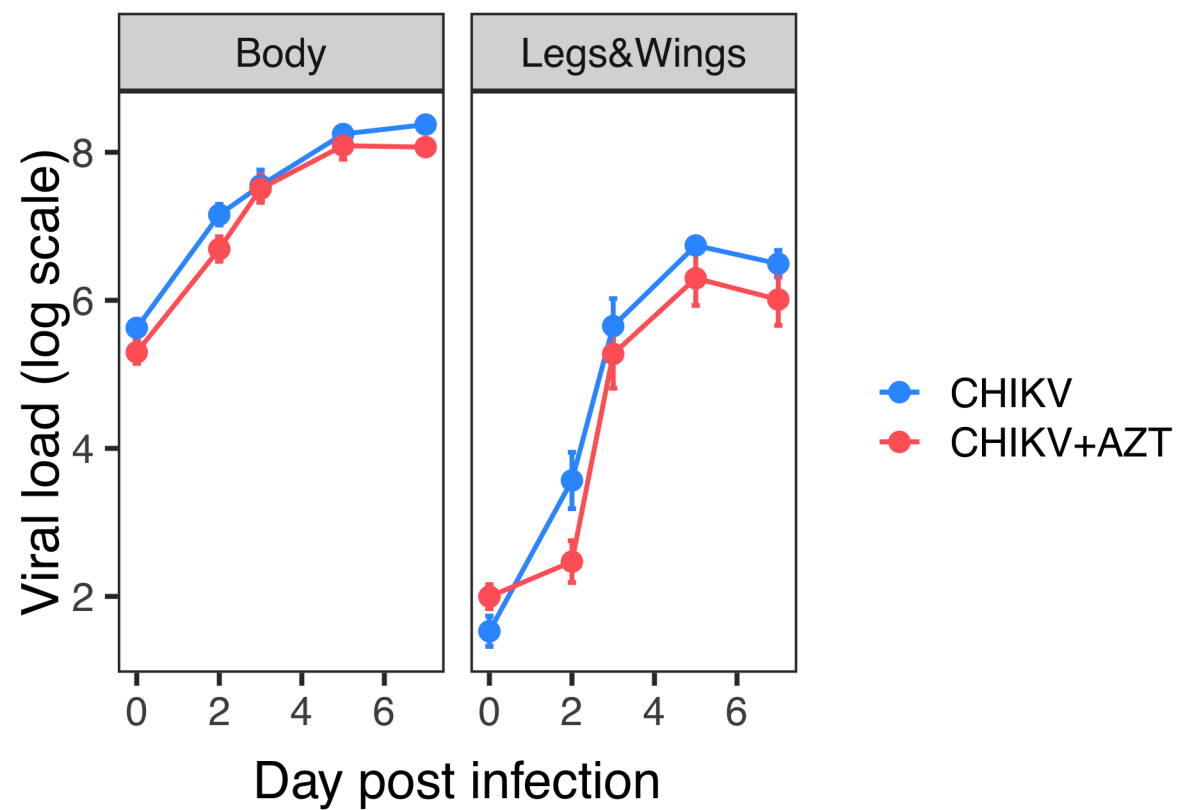


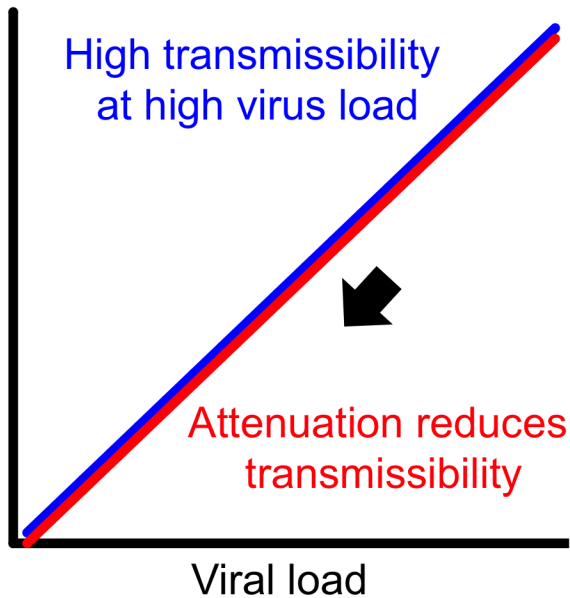
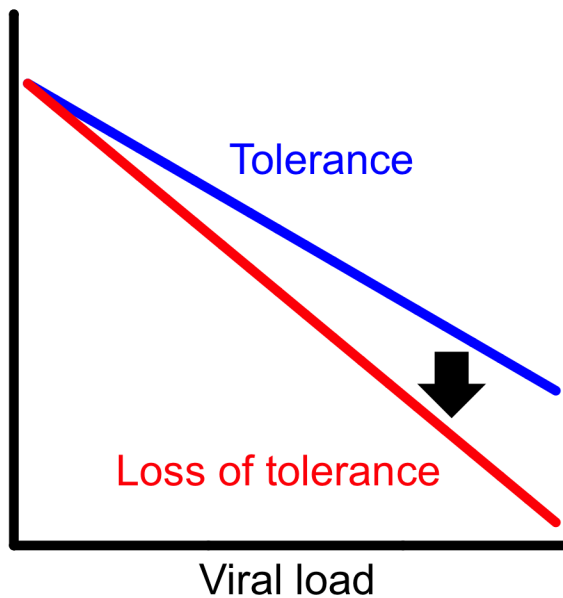
Consequences of reducing tolerance

Short term: stop transmission

Long term: virus attenuation to allow mosquito survival



A**B**

AVirus transmission rate (β)**B**Vector lifespan (α)**C**Virus fitness ($= \alpha \times \beta$)