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## **1** Manipulating mosquito tolerance for arbovirus control

- 2 Louis Lambrechts<sup>1\*</sup> and Maria-Carla Saleh<sup>2\*</sup> 3 4 5 6 <sup>1</sup>Institut Pasteur, Insect-Virus Interactions Unit, Department of Virology, UMR2000, 7 CNRS, 75015 Paris, France 8 <sup>2</sup>Institut Pasteur, Viruses and RNA Interference Unit, Department of Virology, UMR3569, 9 CNRS, 75015 Paris, France \*Corresponding authors (LL: louis.lambrechts@pasteur.fr; MCS : 10 11 carla.saleh@pasteur.fr) 12 13 14 Abstract 15 The inexorable emergence of mosquito-borne arboviruses and the failure of traditional 16 17 vector control methods to prevent their transmission have triggered the development of alternative entomological interventions to render mosquito populations incapable of 18 19 carrying arboviruses. Here, we use a theoretical framework to argue that decreasing mosquito tolerance to arbovirus infection could be a more evolutionarily sustainable 20 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 mosquitoattenuated arbovirus variants that are less transmissible. 24
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### 26 Resistance, tolerance, and the control of mosquito-borne arboviruses

In every host-pathogen relationship, the host can survive by employing two conceptually 28 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 by Albers, who distinguished 'resistance', defined as the ability to suppress establishment 35 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).

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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 slope of host fitness against infection intensity. In the 2000s, animal parasitologists 43 44 borrowed this approach to measure tolerance (the reaction norm of host health to pathogen burden) in a rodent malaria model (Raberg et al., 2007). This landmark study 45 46 demonstrated the existence of genetic variation in tolerance and a genetic trade-off between resistance and tolerance in an animal host-pathogen system. Genetic variation 47 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 importance, and the extent to which their genetic basis is shared, can result in a variety 50 51 of evolutionary trajectories for both the host and the pathogen (Svensson and Raberg, 52 2010).

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

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

- leading to the death of infected vectors- could be a more evolutionarily sustainable
   arbovirus control strategy than increasing mosquito resistance (Figure 1).
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#### 81 What determines mosquito tolerance to arbovirus infection?

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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 infected for the rest of its life. Despite active replication and high viral loads, however, 85 arboviral infections in mosquitoes typically do not result in severe fitness defects. 86 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 experimental evidence for arbovirus virulence in mosquitoes (e.g., (Ciota et al., 2011)). 89 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.

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How do mosquitoes cope with viral infection? Response to infection does not simply consist of activating immune pathways, it also encompasses a broad range of physiological consequences including metabolic adaptations, stress responses, and tissue repair. Critically, upon infection, the homeostatic regulation of these pathways is altered. However, such alterations do not always result in increased disease severity or 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 found in Drosophila, where the transcription factor CrebA is induced upon bacterial 105 106 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 expressed in the fat body, an organ analogous to the mammalian liver and adipose 108 109 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 tolerance modulates host physiology to prevent the deleterious effect of infection-112 113 associated cellular stress.

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

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

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

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

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

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

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 activity and the resulting viral DNA is necessary for mosquito tolerance. Preventing viral 160 161 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 following arbovirus infection in the absence of viral DNA provides the proof of principle 163 164 that infected mosquitoes can be selectively eliminated through the loss of tolerance.

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166 The rationale behind reducing mosquito tolerance rather than increasing resistance to arbovirus infection is that loss of tolerance is expected to be more evolutionarily 167 sustainable. According to evolutionary theory, reducing tolerance should select for 168 169 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 results from a lower evolutionary optimum for viral load, which is also predicted to result 172 173 in lower transmissibility due to the expected positive relationship between viral load and 174 transmission rate (Figure 3).

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The evolutionary response to an intervention that targets mosquito-arbovirus interactions can be examined from both the mosquito and the arbovirus perspectives. There are two reasons for which the evolutionary response of mosquitoes is unlikely to undermine an intervention due to the arbovirus. First, as mentioned above, the deleterious effects of arbovirus infection are generally modest and the mosquito fitness is largely maintained. 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 mosquitoes randomly collected in a highly endemic region of Thailand (Yoon et al., 2012). 183 184 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 reasonable to suggest that arboviruses do not represent a major selective pressure 186 187 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.

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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. Natural selection will thus favor arbovirus mutants that can restore a higher transmission 200 201 rate by evading or suppressing the resistance mechanism. It is worth noting that recent developments in genetic engineering strategies, such as simultaneously targeting 202 203 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 mosquito tolerance, the transmission rate will remain unchanged because, by definition, 205 the viral load is unchanged. However, the duration of transmission will decrease because 206 207 infected vectors have a shortened lifespan. In that case, natural selection will favor

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

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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 mosquito resistance and tolerance will not result in major fitness costs in the absence of 219 220 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 infection by insect-specific viruses. Wolbachia transfection in Ae. aegypti, for example, 224 225 suppresses replication of some insect-specific viruses (Schnettler et al., 2016), and possibly enhances that of others (Amuzu et al., 2018). It is likely that natural tolerance 226 227 mechanisms act across a broad spectrum of viruses. Therefore, specificity against human-pathogenic arboviruses could be achieved by complementing a general but subtle 228 229 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. Resistance and tolerance were found to be genetically correlated in a mouse model 231 (Raberg et al., 2007), reflecting the existence of pleotropic genes that contribute to both 232 233 phenotypes. The endoribonuclease Dicer-2, for example is a cornerstone of mosquito

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

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#### 237 Concluding remarks

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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 as a disease control strategy (Figure 1). In this review, we have provided theoretical 241 242 arguments supporting the idea that reducing tolerance should be more evolutionarily 243 robust than increasing mosquito resistance to arbovirus infection. However, a number of scientific, logistical and ethical challenges remain to be evaluated before converting this 244 idea into a realistic, and sustainable intervention for disease control. Additional 245 246 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 absence of fitness cost for arbovirus-free mosquitoes. Understanding the mechanisms of 250 tolerance will be essential to design a method to safely and specifically manipulate it. 251 Moreover, the mechanism employed to reduce mosquito tolerance will have to be 252 253 delivered and remain effective under field conditions. Delivery may rely on mass releases of gene drive systems that are being developed for other candidate methods of vector 254 255 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 entomological intervention is ultimately established through large-scale field trials with 258 259 epidemiological endpoints. Such a difficult and time-consuming process makes it crucial

to preferentially invest in candidate disease control strategies that are evolutionarilysustainable.

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416

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

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

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

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

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



Α



Β

