



HAL
open science

Arbovirus impact on mosquito behavior: the jury is still out

Théo Maire, Louis Lambrechts, Felix J.H. Hol

► **To cite this version:**

Théo Maire, Louis Lambrechts, Felix J.H. Hol. Arbovirus impact on mosquito behavior: the jury is still out. *Trends in Parasitology*, 2024, 40 (4), pp.292-301. 10.1016/j.pt.2024.02.004 . pasteur-04572547

HAL Id: pasteur-04572547

<https://pasteur.hal.science/pasteur-04572547>

Submitted on 11 May 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Arbovirus impact on mosquito behavior: the jury is still out**

2

3 Théo Maire¹, Louis Lambrechts^{1#}, Felix J. H. Hol^{2*}

4 ¹Institut Pasteur, Université Paris Cité, CNRS UMR2000, Insect-Virus Interactions Unit, Paris, France

5 ²Radboud University Medical Center, Department of Medical Microbiology, Nijmegen, the Netherlands

6 *Corresponding author: felix.hol@radboudumc.nl (F. J. H. Hol)

7 #ORCID number: 0000-0001-5958-2138

8

9 **Keywords:** mosquito behavior; arbovirus; host seeking; blood feeding; host manipulation;
10 transmission dynamics

11

12 **Abstract**

13 Parasites can manipulate host behavior to enhance transmission, but our understanding of
14 arbovirus-induced changes in mosquito behavior is limited. Here, we explore current
15 knowledge on such behavioral alterations in mosquito vectors, focusing on host-seeking and
16 blood-feeding behaviors. Reviewing studies on dengue, Zika, La Crosse, Sindbis, and West Nile
17 viruses in *Aedes* or *Culex* mosquitoes, reveals subtle yet potentially significant effects.
18 However, assay heterogeneity and limited sample sizes challenge definitive conclusions. To
19 enhance robustness, we propose using deep-learning tools for automated behavior
20 quantification and stress the need for standardized assays. Additionally, conducting longitudinal
21 studies across the extrinsic incubation period and integrating diverse traits into modeling
22 frameworks are crucial for understanding the nuanced implications of arbovirus-induced
23 behavioral changes for virus transmission dynamics.

24 The wondrous world of parasite-induced changes in host behavior

25 Parasitic organisms have evolved intricate strategies to optimize their transmission. The adaptive
26 modification of host behavior is a fascinating example of this. Certain **parasites** (see **Glossary**) exhibit
27 the remarkable ability to alter the behavior of their hosts to increase the likelihood of transmission to the
28 next host in their life cycle [1–3]. Such host ‘**manipulation**’ can manifest in various ways, from altering
29 the host’s behavioral patterns, to modifying its physiology or development. Examples abound: the
30 zombie-ant fungus manipulates ant movement to enhance dispersal [4]; gordian worms make crickets
31 jump into the water [5]; rabies virus enhances salivation and aggressiveness [6]; while certain flatworm
32 infections cause grotesque deformations in frogs making them an easy prey for the bird species that are
33 the worm's final host [7]. Parasites may alter their hosts in a multitude of ways, which ultimately may
34 serve the parasite’s reproductive interests – often at the expense of the host. Not all parasite-induced
35 behavioral changes are adaptive, though, because infection side-effects can also inadvertently modify
36 host behavior without increasing parasite fitness [1–3].

37 Insect vectors are no strangers to parasite-induced changes in host physiology and behavior [1,8]. A
38 notable example is *Leishmania* transmission by the sand fly *Lutzomyia longipalpis*. An impressive body
39 of literature has dissected the molecular, physiological, and anatomical impact of *Leishmania* infection
40 providing detailed insights into how the parasite manipulates its sand fly host, and the mechanisms
41 through which this enhances transmission [9–12]. In the context of mosquito-**pathogen** interactions, the
42 behavioral impact of *Plasmodium* infection on *Anopheles* has received considerable attention.
43 Laboratory studies demonstrated that *Plasmodium*-infected mosquitoes more readily engage in **blood**
44 **feeding**, although these changes are likely a combination of general responses to infection and specific
45 effects of the *Plasmodium* parasite [13–18]. Whether *Plasmodium*-induced changes in the mosquito host
46 ultimately lead to enhanced transmission in the field remains a topic of debate, as is evident from
47 publications with strikingly similar titles except for the word 'no', relating to the presence/absence of
48 evidence to support this hypothesis [19,20].

49

50 Are arboviruses of global concern evil masterminds controlling their
51 mosquito hosts?

52 Relatively few studies investigated the effect of arthropod-borne virus (arbovirus) infection on mosquito
53 behavior. Given the abundant results in *Plasmodium*-infected mosquitoes and other examples of host
54 modification by parasites, it has been assumed that arboviruses are also likely to modify the behavior of
55 their vectors. However, considering the significant differences between the infection process and
56 associated host immune responses for viruses and *Plasmodium* parasites, and the large evolutionary
57 distance between the mosquito species that vector arboviruses versus *Plasmodium*, testing this
58 hypothesis requires arbovirus-specific evidence quantifying the impact of infection on mosquito
59 behavior.

60 A variety of behavioral traits along the blood-feeding trajectory may be affected by arboviruses and
61 potentially impact their transmission (**Figure 1, key figure**). In the earliest phase of the behavioral
62 sequence leading to a blood meal, a mosquito needs to find a host, a process known as **host seeking**.
63 Changes in the rate at which mosquitoes engage in host-seeking behavior (e.g. switching from resting
64 to flight) could impact virus transmission by altering host contact, potentially decreasing contact at pre-
65 transmissible stages, while increasing activity when the pathogen has reached the salivary glands (as
66 may be the case in *Plasmodium*-infected mosquitoes [14]). In addition to altering the propensity to
67 engage in host-seeking behavior, host preferences could also be modified to bias the **host preference** of
68 promiscuous biters to hosts that maximize onward transmission of the virus.

69 After alighting on a host, mosquitoes display a variety of behaviors related to blood feeding that may
70 have a profound impact on virus transmission. Mosquitoes may probe the host skin without taking a
71 blood meal, yet potentially transmitting virus, or take a single large blood meal versus multiple smaller
72 meals. Scenarios that do not lead to a full blood meal are likely to result in increased host seeking at a
73 later stage, which could lead to additional virus transmission events.

74 The behavioral subroutines mentioned above may be impacted by virus infection directly, for example
75 through a factor of viral origin, or indirectly, due to the physiological consequences of infection. Both
76 direct and indirect effects on behavior may influence viral fitness (onward virus transmission) and

77 therefore have adaptive value. Direct changes that are adaptive are the purest form of host manipulation
78 by a parasite, yet indirect effects can also have important consequences for human infections and are
79 therefore of equal epidemiological relevance. In this paper, we discuss changes in mosquito behavior
80 that may impact virus transmission, regardless of whether these changes are 'by products' of infection,
81 or direct manipulations effectuated by the virus.

82 Due to the paucity of empirical evidence and the heterogeneous nature of behavioral data, our knowledge
83 of the extent to which arbovirus infections modify mosquito behavioral traits is patchy. Here, we review
84 studies on dengue virus (DENV), Zika virus (ZIKV), La Crosse virus (LACV), Sindbis virus (SINV)
85 and West Nile virus (WNV) infecting adult *Aedes* or *Culex* mosquitoes, with a focus on blood-feeding
86 and host-seeking behaviors. The threat these arboviruses pose to approximately half the global human
87 population confers high societal relevance to fundamental studies regarding the biological phenomena
88 that drive virus transmission [21–23]. There are several other arboviruses of epidemiological
89 significance, such as yellow fever and chikungunya viruses, yet to our knowledge the impact of these
90 viruses on mosquito behavior has not been studied.

91 Our analysis of the existing literature reveals that the effects of arbovirus infections on mosquito
92 behavior are likely subtle (**Table 1**). Drawing general conclusions is hampered by the diversity of
93 behavioral assays being used in different laboratories (complicating comparisons and/or meta-analysis)
94 and weak statistical support for the conclusions drawn in some studies. Nevertheless, taking the currently
95 available literature as a whole suggests that certain arboviruses do indeed impact mosquito behavior, in
96 a manner that may have meaningful consequences for virus transmission.

97

98 Empirical evidence is blurred by inconsistent assays, small sample sizes,
99 and likely subtle effects

100 The available literature displays a striking diversity of assays and approaches used to assess aspects of
101 mosquito host-seeking and blood-feeding behaviors. Assays range from the direct visual inspection of
102 mosquitoes feeding on a live host (e.g. in [24]), to long-term monitoring of activity patterns using assays
103 originally developed for fruit flies (e.g. in [25]). Another general theme is the relatively small sample

104 size of experiments, with many studies reporting on cohorts of 10-50 mosquitoes per condition. In
105 conjunction with the significant behavioral heterogeneity that is often observed between individual
106 insects, the limited sample sizes of many studies impact the statistical power and generalizability of
107 results, seducing some authors to relax statistical rigor and draw conclusions that seem only partly
108 supported by the data.

109

110 *Host-seeking behavior*

111 The few studies that investigated host-seeking behavior at the long range (1 to 2 meter) reported similar
112 trends across different mosquito-arbovirus pairs: both WNV and LACV reduced host-seeking activity
113 of their mosquito hosts, *Culex pipiens* and *Aedes triseriatus*, respectively [26,27]. Both studies assayed
114 host seeking using an olfactometer at time points beyond the **extrinsic incubation period** (i.e. when the
115 virus has become transmissible) and observed behavioral changes that would potentially reduce virus
116 transmission. Interestingly, this effect is opposite to the impact of *Plasmodium* infection on the long-
117 range attraction of *Anopheles gambiae* [17]. WNV did not affect antennal olfactory responsiveness,
118 suggesting that the reduced host-seeking response was not caused by an altered response to volatile host
119 cues. Vogels *et al.* furthermore considered the possibility that WNV could induce a shift in the host
120 preference of infected *Cx. pipiens* mosquitoes, possibly enhancing attraction to birds while reducing
121 attraction to dead-end hosts, yet no shift in preference was observed [26].

122 General activity patterns have also been used as a proxy to quantify the host-seeking behavior of
123 mosquitoes. In studies comparing DENV- or ZIKV-infected and uninfected *Aedes aegypti*, locomotor
124 activity was continuously monitored for 2 weeks [25,28,29]. For both viruses, infection-stage-specific
125 changes in activity patterns were reported, however, the assays used did not allow flight and constrained
126 animal movement (mosquitoes were housed in 7-millimeter diameter tubes) leaving doubts whether the
127 observed activity patterns are a good proxy for host-seeking behavior. In search of a neuro-sensory
128 mechanism driving the observed changes in activity patterns, an increased sensitivity for human odors
129 was observed in the antennae of DENV-infected *Ae. aegypti* [29]. This finding contrasts observations
130 with WNV-infected *Cx. pipiens*, which did not show elevated sensitivity to host odors [26], and raises

131 the interesting possibility that DENV may enhance host-seeking behavior, something that could be tested
132 in an **olfactometer**. These contrasting observations in mosquito species from the same subfamily
133 (Culicinae) infected with arboviruses from the same genus (*Flavivirus*) highlight the striking differences
134 that can be observed in related systems.

135

136 *Blood-feeding behavior*

137 The later stages of the behavioral sequence leading to a blood meal, including short-range attraction (<
138 1 meter), landing, **biting**, **probing**, and **engorging** have received more scrutiny, especially in the case
139 of DENV-infected *Ae. aegypti*. Several studies characterized DENV-infected mosquitoes blood feeding
140 on mice 14 days post mosquito infection. Four studies reported no differences in the duration of probing
141 or engorging time [30–33]. These results are seemingly in contrast with a recent study [24] by Xiang *et al.*,
142 which claimed decreased probing efficiency and increased duration of probing before blood
143 ingestion. Reduced probing efficiency would be especially meaningful since Xiang *et al.* demonstrated
144 that brief probing bouts that do not lead to engorgement, are an efficient means to transmit DENV in a
145 mouse model. However, Xiang *et al.* reported behavioral differences between infected and non-infected
146 mosquitoes whose statistical significance was close but did not reach the conventional threshold of <5%
147 false positive rate in a multiple factor analysis (statistical significance was only obtained with single-
148 sided tests in univariate analyses). The frailty of statistical support for these effects, together with the
149 opposing results of earlier studies, call for caution in reaching a conclusion. Future studies involving
150 larger cohorts of mosquitoes are required to come to a statistically robust verdict on the effect of DENV
151 infection on *Ae. aegypti* biting behavior.

152 The effect of SINV infection on *Ae. aegypti* blood-feeding behavior was studied in groups of 20
153 mosquitoes with a systemic virus **dissemination**, which displayed increased feeding time and larger
154 blood meals compared to infected mosquitoes without virus dissemination [34]. Qualls *et al.*, however,
155 only report on the behaviors of mosquitoes that effectively engage in blood feeding. The same is true
156 for studies on DENV-infected *Ae. aegypti* – only a subset of studies analyzes blood-feeding behavior at
157 the population level, and in this case, does not observe significant differences in the fraction of

158 mosquitoes obtaining a blood meal [24,32]. Considering the impact of arbovirus infection at both the
159 individual and population level, however, is crucial to fully appreciate the implications for virus
160 transmission. If, for example, a virus would increase the probing behavior of a mosquito after alighting
161 on a host, yet the same virus would decrease the activation rate (i.e. lower the population fraction that
162 engages in host-seeking behavior in the first place) these effects would have opposing consequences for
163 virus transmission. LACV currently is the only arbovirus for which the effects on mosquito activation,
164 long-range attraction, and biting behavior have been quantified together [27,35,36]. Modeling
165 approaches enabling the integration of these data to quantify the overall effect on virus transmission are
166 a very interesting avenue of future research.

167 It is important to note that most studies report on cohorts of <20 mosquitoes, with the largest studies
168 analyzing just over a hundred individuals. Behavioral traits often show considerable variation, even
169 among individual insects sharing the same genotype and reared under identical conditions [37]. The
170 expected behavioral heterogeneity, together with limited sample sizes, present the need for future studies
171 that quantify the impact of arboviruses on blood-feeding behavior in larger groups of mosquitoes using
172 accurate, high-throughput behavioral assays. In addition to the basic need for statistically robust studies,
173 standardization of behavioral assays would facilitate comparisons between experiments carried out by
174 different research groups and/or performed on different mosquito-arbovirus pairs (e.g. [38]).
175 Standardized approaches furthermore present the exciting possibility to uncover whether specific
176 behavioral changes are particular to specific mosquito-arbovirus combinations, or if general themes exist
177 in the way viruses impact the behavior of their mosquito hosts. Such an effort could be kick-started
178 through comparative studies on arboviruses from the same genus infecting the same mosquito species
179 (e.g. DENV and ZIKV), to ultimately compare arboviruses from different viral families infecting
180 mosquitoes from different genera. Likewise, the eco-evolutionary forces that shape the behavioral
181 impact of viruses may be explored by comparing viruses vectored by mosquitoes exploiting
182 similar/different ecological niches. Careful consideration of the ecology of the virus and mosquito may
183 lead to specific hypotheses regarding which behavioral changes in the mosquito may maximize virus
184 transmission. For instance, the diversity and density of available blood meal hosts, or the time of day at
185 which mosquitoes feed may lead to different outcomes (and thus different fitness effects) for the same

186 change in behavior. Performing such studies at scale necessitates high-throughput behavioral assays
187 facilitating automated quantification of behavior, yet all currently available studies use human observers
188 to score behaviors.

189

190 Monitoring the dynamics of behavioral changes throughout the
191 extrinsic incubation period

192 An important direction to improve our understanding of the effect of arbovirus infection on mosquito
193 behavior involves the evaluation of behavioral changes throughout the entire extrinsic incubation period
194 (i.e. longitudinal studies over weeks after the infectious blood meal). Earlier studies primarily focused
195 on assessing the host-seeking or blood-feeding behaviors of mosquitoes at least 2 weeks after the
196 infectious blood meal, in order to exceed the typical duration of the extrinsic incubation period (e.g. 10
197 days for DENV at 25-30°C [39]). This timing ensures that the virus has reached the salivary glands and
198 is readily transmissible upon biting. If the virus actively modifies mosquito behavior, one expects
199 behavioral changes enhancing transmission at the expense of increased mosquito mortality, such as a
200 higher probing rate or a smaller blood meal size, to occur only after the virus becomes transmissible
201 (**Figure 1**). Such stage-dependent manipulation has been observed for *Plasmodium* parasites, with
202 opposing effects on behavior according to the mosquito's infection status (see Figure 1 in ref. [15]).
203 However, these differential effects correspond to different developmental stages of the parasite, which
204 lack an equivalent in arboviruses. For arboviruses, the kinetics of dissemination can substantially vary
205 as function of the viral titer in the blood meal [40] and the virus strain [41]. Observing opposite or at
206 least distinct effects of arbovirus infection on blood-feeding behavior before, and after the virus becomes
207 transmissible would support the hypothesis of a direct behavioral modification, rather than a non-
208 specific physiological response to infection.

209

210 Integrating different traits in a modeling framework to assess the
211 potential impact on virus transmission

212 To estimate the potential epidemiological consequences of arbovirus-induced behavioral changes in
213 mosquitoes, it is crucial to incorporate all relevant traits into a comprehensive modeling framework.
214 While extensive experiments are needed to confirm the amplitude of the behavioral changes induced by
215 arbovirus infection, it is reasonable to speculate that if the effect were large, it would have been
216 documented already.

217 The question arises whether relatively minor changes observed under laboratory conditions could have
218 epidemiologically meaningful implications for the dynamics of arbovirus transmission in nature, and if
219 such information can be used to evaluate the risk of arbovirus outbreaks. Theoretical frameworks to
220 estimate the force of infection of mosquito-borne diseases, generally extensions of the Ross-Macdonald
221 model, incorporate mosquito behavior as a single trait called "biting rate per person per day" [42,43].

222 To better quantify the epidemiological consequences of arbovirus-induced behavioral changes, novel
223 modeling efforts that integrate multiple behavioral traits along the blood-feeding trajectory (that may be
224 differently affected by infection) are necessary. Integrating such efforts with detailed models of the
225 metabolic interplay between mosquitoes and pathogens, as was recently done for *Plasmodium* [44],
226 would be especially powerful to quantify the multi-dimensional effects pathogen infections have on the
227 life history of mosquitoes. Alternatively, models that appropriately condense the intricate steps of host-
228 seeking and blood-feeding behaviors (activation, landing, probing, engorging) into a coarse-grained
229 "biting rate per person per day" could be developed. We furthermore note that there currently are no
230 data on the impact of arbovirus infections on several aspects of mosquito behavior that are relevant for
231 virus transmission, for example host seeking beyond the 2-meter range in real-world scenarios that are
232 navigationally more complex than laboratory settings.

233 The force of infection also depends on mosquito survival, which seems to be modestly decreased by
234 arbovirus infection [38]. Interestingly, a mathematical model of *Plasmodium* infection suggested that

235 behavioral changes could potentially enhance the survival of infected mosquitoes during the pre-
236 transmissible phase, leading to an increase in the force of infection [13]. However, no such models or
237 empirical data exist for arboviruses infection, highlighting the need for future work to assess the
238 importance of infection on mosquito survival in field conditions (including the risk associated with blood
239 feeding on live hosts).

240 A critical factor in extrapolating laboratory results to field-relevant conditions is recognizing that
241 mosquito biting rates may vary significantly based on the hour of the day, local habitats, mosquito
242 strains, and, notably, individual mosquitoes [45]. In the context of a biting rate that varies widely even
243 in the absence of virus infection, an average subtle effect of virus infection might be overwhelmed by
244 other factors and therefore may not be directly relevant to the dynamics of virus transmission.
245 Conversely, a modest average increase in biting rate due to infection might conceal "extreme" behaviors
246 of a few infected individuals. The contribution of inter-individual variability in mosquito behavior and
247 the existence of potential "super-spreaders" [46] has yet to be established for arboviruses but could
248 profoundly alter the predicted effects of infection on mosquito behavior. The real-world relevance of
249 laboratory findings furthermore depends on how closely the model system aligns with biological reality,
250 it is therefore important to consider the effect of laboratory adaptation for the observed phenotypes and
251 the significant genetic, ecological, and phenotypic variation between different (geographical) variants
252 of the same species [47].

253

254 Towards a solid empirical foundation by leveraging the raw power of
255 deep learning and the machine vision toolbox

256 Solid empirical data are a key necessity to fully appreciate the impact of arbovirus infection on mosquito
257 behavior, and its epidemiological consequences. Rather than merely replicating previous experiments
258 with a larger number of mosquitoes, a powerful approach to gather large and robust data sets involves
259 leveraging the expanding array of tools based on **deep learning**. Indeed, most of the studies mentioned
260 above, including the most recent ones, rely entirely on manual annotation of videos of mosquitoes to

261 extract quantities such as probing time or the number of re-feeding attempts. These conventional
262 approaches, apart from being time-consuming, rely on subjective human judgment that is challenging
263 to replicate beyond the original laboratory setting. Automatic behavioral quantification directly from
264 video data, however, is currently gaining traction for organisms as diverse as the unicellular predator
265 *Lacrymaria olor* [48], collectively foraging desert locusts [49] and cricket-hunting marmosets [50].
266 Deep-learning-based supervised [51,52] and unsupervised methods [53] allow automatic tracking of
267 insect body parts in videos of multiple insects navigating experimental arenas. Efforts in this direction
268 have been successfully applied to characterize the biting behavior of a variety of mosquito species blood
269 feeding on artificial host mimics [54,55]. As various mosquito appendages integrate different
270 environmental stimuli through contact dependent sensing [56], detailed body part tracking allows the
271 study of stimulus-response relationships (e.g. touch based avoidance of repellants [54]) and may thus
272 be used to determine if virus infection augments such responses. Body part tracking can furthermore be
273 used to estimate the pose of an animal (the geometric configuration of all its body parts). As behaviors
274 often consist of a sequence of stereotyped motions, the dynamics of an animal's pose can be used to
275 automatically assign short stretches of motion sequences to behavioral classes enabling statistical
276 assessment of behavioral patterns [57,58]. These approaches have enhanced our understanding of fruit
277 fly behavior [57,59], and in combination with custom-built experimental arenas eliciting relevant
278 mosquito behaviors, will be a powerful approach to quantify the behavioral impact of arboviruses on
279 mosquito behavior.

280

281 Concluding remarks and future perspectives

282 Mosquito behavioral changes induced by arboviruses have fascinated researchers for decades. Our
283 synthesis of the literature suggests that such changes are likely subtle in magnitude, yet they may
284 nevertheless impact the spread of mosquito-borne viruses. The molecular mechanisms through which
285 virus infections modify mosquito behavior, and the evolutionary processes that shape those mechanisms,
286 present an array of exciting **outstanding questions**. Future research in this direction may benefit from
287 comparative analysis between different mosquito-virus systems and other mosquito-borne pathogens,

288 such as *Plasmodium*. In addition to dissecting the molecular mechanisms driving arbovirus-induced
289 behavioral changes in mosquitoes, we argue that systematic studies with appropriate statistical power
290 are necessary to move arbovirus-induced behavioral changes from the realm of "an exciting yet
291 controversial hypothesis", to a biological phenomenon for which there is (or is not) a sound empirical
292 basis. Establishing this foundation will require accurate, standardized, and high-throughput assays to
293 quantify mosquito behavior. We anticipate that novel behavioral assays that leverage advances in
294 computer vision and deep learning will improve the accuracy and robustness of behavioral
295 measurements, paving the way towards a sound verdict regarding the impact arboviruses may have on
296 mosquito behavior.

297

298

299 **Acknowledgements**

300 This work was supported by the French Government's Investissement d'Avenir program Laboratoire
301 d'Excellence Integrative Biology of Emerging Infectious Diseases (grant ANR-10-LABX-62-IBEID),
302 program Institut Convergences INCEPTION (grant ANR-16-CONV-0005), a VIDI (grant
303 VI.Vidi.213.167) from NWO (the Dutch science foundation), and a Radboudumc Hypatia fellowship.

304

305 **Declaration of interests**

306 The authors declare no competing interests.

307

308 **References**

- 309 1. Hurd, H. (2003) Manipulation of Medically Important Insect Vectors by Their Parasites. *Annu.*
310 *Rev. Entomol.* 48, 141–161
- 311 2. Lefèvre, T. *et al.* (2009) The ecological significance of manipulative parasites. *Trends Ecol.*
312 *Evol.* 24, 41–48
- 313 3. Moore, J. (2002) *Parasites and the behavior of animals*, Oxford University Press
- 314 4. Hughes, D.P. *et al.* (2011) Behavioral mechanisms and morphological symptoms of zombie
315 ants dying from fungal infection. *BMC Ecol.* 11, 13
- 316 5. Thomas, F. *et al.* (2002) Do hairworms (Nematomorpha) manipulate the water seeking

317 behaviour of their terrestrial hosts? *J. Evol. Biol.* 15, 356–361

318 6. Hueffer, K. *et al.* (2017) Rabies virus modifies host behaviour through a snake-toxin like
319 region of its glycoprotein that inhibits neurotransmitter receptors in the CNS. *Sci. Rep.* 7, 12818

320 7. Johnson, P.T.J. *et al.* (1999) The Effect of Trematode Infection on Amphibian Limb
321 Development and Survivorship. *Science* 284, 802–804

322 8. Lefèvre, T. and Thomas, F. (2008) Behind the scene, something else is pulling the strings:
323 Emphasizing parasitic manipulation in vector-borne diseases. *Infect. Genet. Evol.* 8, 504–519

324 9. Rogers, M.E. *et al.* (2008) Leishmania chitinase facilitates colonization of sand fly vectors and
325 enhances transmission to mice. *Cell. Microbiol.* 10, 1363–1372

326 10. Serafim, T.D. *et al.* (2021) Leishmaniasis: the act of transmission. *Trends Parasitol.* 37, 976–
327 987

328 11. Rogers, M.E. and Bates, P.A. (2007) Leishmania Manipulation of Sand Fly Feeding Behavior
329 Results in Enhanced Transmission. *PLoS Pathog.* 3, e91

330 12. Yanase, R. *et al.* (2023) Formation and three-dimensional architecture of Leishmania adhesion
331 in the sand fly vector. *eLife* 12, e84552

332 13. Cator, L.J. *et al.* (2012) Do malaria parasites manipulate mosquitoes? *Trends Parasitol.* 28,
333 466–470

334 14. Cator, L.J. *et al.* (2013) ‘Manipulation’ without the parasite: altered feeding behaviour of
335 mosquitoes is not dependent on infection with malaria parasites. *Proc. R. Soc. B Biol. Sci.* 280,
336 20130711

337 15. Cator, L.J. *et al.* (2015) Immune response and insulin signalling alter mosquito feeding
338 behaviour to enhance malaria transmission potential. *Sci. Rep.* 5, 11947

339 16. Murdock, C.C. *et al.* (2017) Immunity, host physiology, and behaviour in infected vectors.
340 *Curr. Opin. Insect Sci.* 20, 28–33

341 17. Smallegange, R.C. *et al.* (2013) Malaria Infected Mosquitoes Express Enhanced Attraction to
342 Human Odor. *PLoS ONE* 8, e63602

343 18. Stanczyk, N.M. *et al.* (2017) Effects of malaria infection on mosquito olfaction and behavior:
344 extrapolating data to the field. *Curr. Opin. Insect Sci.* 20, 7–12

345 19. Vantaux, A. *et al.* (2015) Host-seeking behaviors of mosquitoes experimentally infected with
346 sympatric field isolates of the human malaria parasite *Plasmodium falciparum*: no evidence for host
347 manipulation. *Front. Ecol. Evol.* 3

348 20. Vantaux, A. *et al.* (2021) Field evidence for manipulation of mosquito host selection by the
349 human malaria parasite, *Plasmodium falciparum*. *Peer Community J.* 1, e13

350 21. Kraemer, M.U.G. *et al.* (2019) Past and future spread of the arbovirus vectors *Aedes aegypti*
351 and *Aedes albopictus*. *Nat. Microbiol.* 4, 854–863

352 22. Messina, J.P. *et al.* (2019) The current and future global distribution and population at risk of
353 dengue. *Nat. Microbiol.* 4, 1508–1515

- 354 23. Ryan, S.J. *et al.* (2019) Global expansion and redistribution of Aedes-borne virus transmission
355 risk with climate change. *PLoS Negl. Trop. Dis.* 13, e0007213
- 356 24. Wei Xiang, B.W. *et al.* (2022) Dengue virus infection modifies mosquito blood-feeding
357 behavior to increase transmission to the host. *Proc. Natl. Acad. Sci.* 119, e2117589119
- 358 25. Lima-Camara, T.N. *et al.* (2011) Dengue infection increases the locomotor activity of Aedes
359 aegypti females. *PloS One* 6, e17690
- 360 26. Vogels, C.B.F. *et al.* (2017) Virus interferes with host-seeking behaviour of mosquito. *J. Exp.*
361 *Biol.* 220, 3598–3603
- 362 27. Yang, F. *et al.* (2019) Effects of La Crosse virus infection on the host-seeking behavior and
363 levels of two neurotransmitters in Aedes triseriatus. *Parasit. Vectors* 12, 397
- 364 28. Gaburro, J. *et al.* (2018) Neurotropism and behavioral changes associated with Zika infection
365 in the vector *Aedes aegypti*. *Emerg. Microbes Infect.* 7, 1–11
- 366 29. Tallon, A.K. *et al.* (2020) Dengue infection modulates locomotion and host seeking in Aedes
367 aegypti. *PLoS Negl. Trop. Dis.* 14, e0008531
- 368 30. Putnam, J.L. and Scott, T.W. (1995) Blood-feeding behavior of dengue-2 virus-infected Aedes
369 aegypti. *Am. J. Trop. Med. Hyg.* 52, 225–227
- 370 31. Platt, K.B. *et al.* (1997) Impact of dengue virus infection on feeding behavior of Aedes
371 aegypti. *Am. J. Trop. Med. Hyg.* 57, 119–125
- 372 32. Sim, S. *et al.* (2012) Dengue virus infection of the Aedes aegypti salivary gland and
373 chemosensory apparatus induces genes that modulate infection and blood-feeding behavior. *PLoS*
374 *Pathog.* 8, e1002631
- 375 33. Maciel-de-Freitas, R. *et al.* (2013) The influence of dengue virus serotype-2 infection on
376 Aedes aegypti (Diptera: Culicidae) motivation and avidity to blood feed. *PloS One* 8, e65252
- 377 34. Qualls, W.A. *et al.* (2012) Sindbis Virus Infection Alters Blood Feeding Responses and DEET
378 Repellency in Aedes aegypti (Diptera: Culicidae). *J. Med. Entomol.* 49, 418–423
- 379 35. Grimstad, P.R. *et al.* (1985) Vector Competence of Aedes Hendersoni (Diptera: Culicidae) for
380 La Crosse Virus and Evidence of a Salivary-Gland Escape Barrier¹. *J. Med. Entomol.* 22, 447–453
- 381 36. Jackson, B.T. *et al.* (2012) La Crosse Virus Infection Alters Blood Feeding Behavior in Aedes
382 triseriatus and Aedes albopictus (Diptera: Culicidae). *J. Med. Entomol.* 49, 1424–1429
- 383 37. Werkhoven, Z. *et al.* (2021) The structure of behavioral variation within a genotype. *eLife* 10,
384 e64988
- 385 38. Lambrechts, L. and Scott, T.W. (2009) Mode of transmission and the evolution of arbovirus
386 virulence in mosquito vectors. *Proc. Biol. Sci.* 276, 1369–1378
- 387 39. Tjaden, N.B. *et al.* (2013) Extrinsic Incubation Period of Dengue: Knowledge, Backlog, and
388 Applications of Temperature Dependence. *PLoS Negl. Trop. Dis.* 7, e2207
- 389 40. Lequime, S. *et al.* (2020) Modeling intra-mosquito dynamics of Zika virus and its dose-
390 dependence confirms the low epidemic potential of Aedes albopictus. *PLOS Pathog.* 16, e1009068

- 391 41. Fontaine, A. *et al.* (2018) Epidemiological significance of dengue virus genetic variation in
392 mosquito infection dynamics. *PLOS Pathog.* 14, e1007187
- 393 42. Smith, D.L. *et al.* (2014) Recasting the theory of mosquito-borne pathogen transmission
394 dynamics and control. *Trans. R. Soc. Trop. Med. Hyg.* 108, 185–197
- 395 43. Cator, L.J. *et al.* (2020) The Role of Vector Trait Variation in Vector-Borne Disease
396 Dynamics. *Front. Ecol. Evol.* 8, 189
- 397 44. Carrillo-Bustamante, P. *et al.* (2023) Evolutionary modelling indicates that mosquito
398 metabolism shapes the life-history strategies of Plasmodium parasites. *Nat. Commun.* 14, 8139
- 399 45. Zahid, M.H. *et al.* (2023) The biting rate of *Aedes aegypti* and its variability: A systematic
400 review (1970–2022). *PLoS Negl. Trop. Dis.* 17, e0010831
- 401 46. Cooper, L. *et al.* (2019) Pareto rules for malaria super-spreaders and super-spreading. *Nat.*
402 *Commun.* 10, 3939
- 403 47. Aubry, F. *et al.* (2020) Enhanced Zika virus susceptibility of globally invasive *Aedes aegypti*
404 populations. *Science* 370, 991–996
- 405 48. Coyle, S.M. *et al.* (2019) Coupled Active Systems Encode an Emergent Hunting Behavior in
406 the Unicellular Predator *Lacrymaria olor*. *Curr. Biol.* 29, 3838–3850.e3
- 407 49. Günzel, Y. *et al.* (2023) Information integration for decision-making in desert locusts. *iScience*
408 26, 106388
- 409 50. Shaw, L. *et al.* (2023) Fast prediction in marmoset reach-to-grasp movements for dynamic
410 prey. *Curr. Biol.* 33, 2557–2565.e4
- 411 51. Mathis, A. *et al.* (2018) DeepLabCut: markerless pose estimation of user-defined body parts
412 with deep learning. *Nat. Neurosci.* 21, 1281–1289
- 413 52. Pereira, T.D. *et al.* (2022) SLEAP: A deep learning system for multi-animal pose tracking.
414 *Nat. Methods* 19, 486–495
- 415 53. Plum, F. *et al.* (2023) replicAnt: a pipeline for generating annotated images of animals in
416 complex environments using Unreal Engine. *Nat. Commun.* 14, 7195
- 417 54. Hol, F.J. *et al.* (2020) BiteOscope, an open platform to study mosquito biting behavior. *eLife*
418 9, e56829
- 419 55. Murray, G.P.D. *et al.* (2023) Characterizing Mosquito Biting Behavior Using the BiteOscope.
420 *Cold Spring Harb. Protoc.* 2023, pdb.prot108176
- 421 56. Baik, L.S. and Carlson, J.R. (2020) The mosquito taste system and disease control. *Proc. Natl.*
422 *Acad. Sci.* 117, 32848–32856
- 423 57. Berman, G.J. *et al.* (2014) Mapping the stereotyped behaviour of freely moving fruit flies. *J.*
424 *R. Soc. Interface* 11, 20140672
- 425 58. Luxem, K. *et al.* (2022) Identifying behavioral structure from deep variational embeddings of
426 animal motion. *Commun. Biol.* 5, 1267
- 427 59. McKenzie-Smith, G.C. *et al.* (2023) Capturing continuous, long timescale behavioral changes

- 428 in *Drosophila melanogaster* postural data. DOI: 10.48550/ARXIV.2309.04044
- 429 60. Zhang, H. *et al.* (2022) A volatile from the skin microbiota of flavivirus-infected hosts
430 promotes mosquito attractiveness. *Cell* 185, 2510-2522.e16
- 431 61. Padilha, K.P. *et al.* (2018) Zika infection decreases *Aedes aegypti* locomotor activity but does
432 not influence egg production or viability. *Mem. Inst. Oswaldo Cruz* 113
- 433 62. Westby, K.M. *et al.* (2016) How do Nutritional Stress and La Crosse Virus Infection Interact?
434 Tests for Effects on Willingness to Blood Feed and Fecundity in *Aedes albopictus* (Diptera:
435 Culicidae). *J. Med. Entomol.* 53, 166–171
- 436 63. Ciota, A.T. *et al.* (2011) The costs of infection and resistance as determinants of West Nile
437 virus susceptibility in *Culex* mosquitoes. *BMC Ecol.* 11, 23
- 438 64. Ciota, A.T. *et al.* (2013) The evolution of virulence of West Nile virus in a mosquito vector:
439 implications for arbovirus adaptation and evolution. *BMC Evol. Biol.* 13, 71
- 440 65. Sylvestre, G. *et al.* (2013) Age-Dependent Effects of Oral Infection with Dengue Virus on
441 *Aedes aegypti* (Diptera: Culicidae) Feeding Behavior, Survival, Oviposition Success and Fecundity.
442 *PLoS ONE* 8, e59933

443

444 **Glossary**

445 **Blood feeding:** Behavior during which a mosquito acquires a blood meal. Blood feeding consists of
446 activation, host seeking, landing on a host, and piercing the host's skin to access blood vessels and
447 ingest blood.

448 **Biting:** Physical act where a mosquito uses its specialized mouthparts to penetrate the host's skin and
449 initiate blood feeding. Often used colloquially to refer to any behavior in which the mosquito
450 mouthparts pierce the skin, including probing and engorging.

451 **Deep learning:** Field of artificial intelligence that employs artificial neural networks with multiple
452 layers to automatically extract intricate features and patterns from data.

453 **Dissemination:** Process by which arboviruses spread to secondary tissues of the mosquito vector after
454 initial infection of the digestive tract through an infectious blood meal, eventually leading to infection
455 of the salivary glands and potential transmission to a new host during subsequent blood feeding.

456 **Engorging:** Process of imbibing blood by which a mosquito becomes partially or fully satiated with
457 blood when feeding on a host.

458 **Extrinsic incubation period:** Time it takes for an arbovirus to propagate within the mosquito vector
 459 after an infectious blood meal, until it reaches the salivary glands, from where it can be transmitted to
 460 a new host during a subsequent blood meal.

461 **Host preference:** Selective tendencies of mosquito vectors in choosing specific host species for blood
 462 feeding.

463 **Host seeking:** Active behavior exhibited by mosquitoes as they search for and locate suitable hosts for
 464 blood feeding.

465 **Manipulation:** Adaptive strategy of parasites to favor the completion of their life cycle by altering the
 466 behavior, physiology, or morphology of their host.

467 **Olfactometer:** Device that delivers controlled odor stimuli to observe and quantify flight-based insect
 468 behaviors, particularly their attraction or orientation to specific scents.

469 **Parasite:** Organism that lives in or on another organism (the host) and derives its nourishment or other
 470 benefits at the host's expense, including specific viruses, bacteria, fungi, protozoa, and multicellular
 471 organisms.

472 **Pathogen:** Biological agent, such as a microorganism or virus, capable of disrupting the normal
 473 physiological functions of the host organism, leading to the manifestation of infectious diseases.

474 **Probing:** Insertion of a mosquito's mouthparts into a host's skin during the initial phase of blood
 475 feeding, prior to imbibing blood.

476

477 **Table 1. Summary of published effects of arbovirus infection on various mosquito behavioral**
 478 **traits related to virus transmission.**

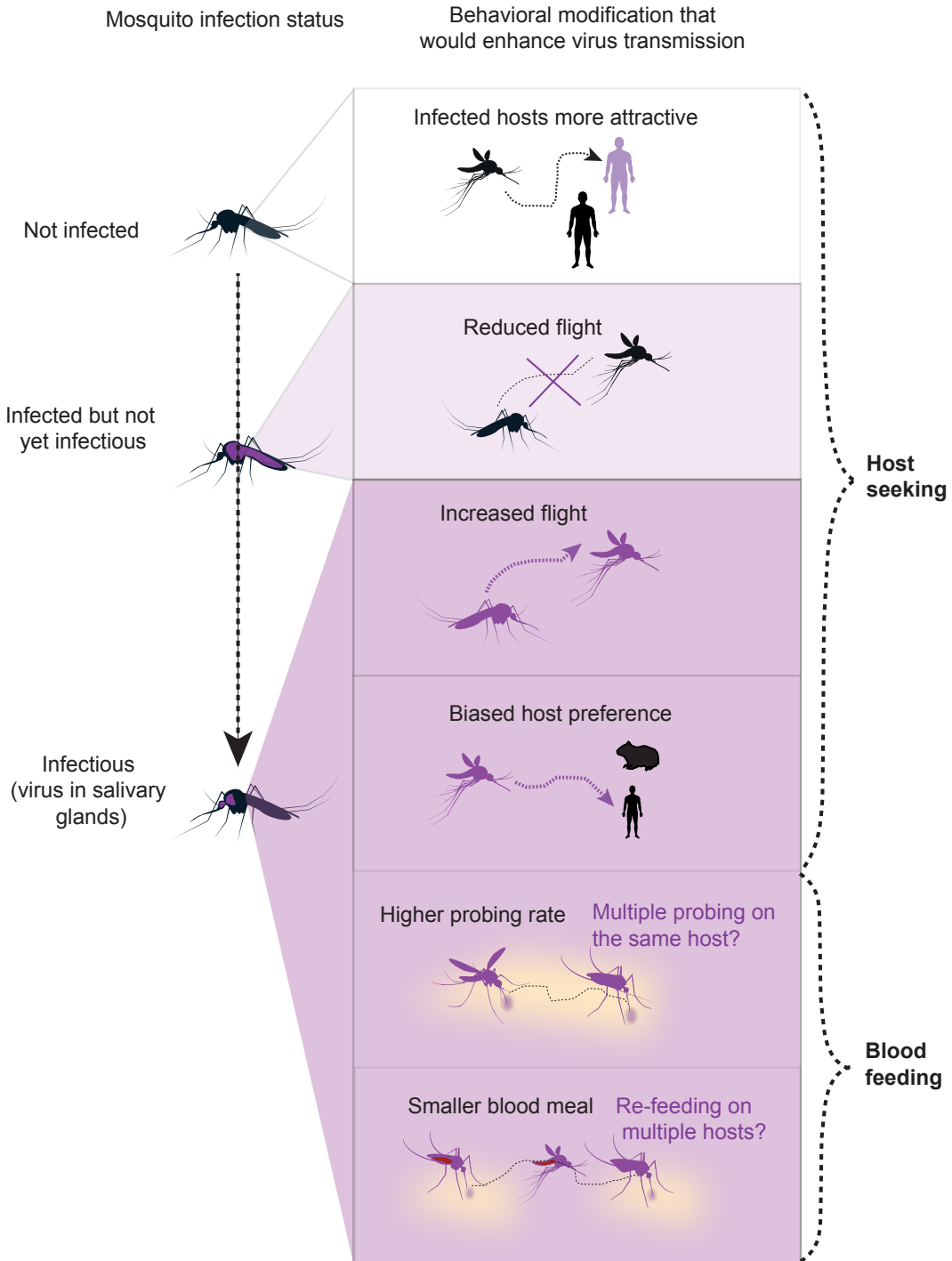
		Arbovirus				
Main behavior	Specific phenotype	DENV	ZIKV	LACV	WNV	SINV
Host seeking	Response to host odors	Increased attractivity [60]	+			

	Flight activity	Increased [25,29] Modified [28]	+ ?	Decreased [61] Modified [28]	- ?				
	Long-range (duration or fraction activated)					Less activation [27]	-	Less activation [26]	-
	Short-range (duration or fraction landing)					No effect [27]	=		Longer time to land [34] -
	Host preference							No effect [26]	=
Blood feeding	Probing (number of probes)	More probing [24] No effect [32]	+ =			No effect [27]	=		No effect [34] =
	Biting (engorgement rate; fraction biting)	Less feeding [33] No effect [24,30,31]	- =			More re-feeding [36,62] No effect [27]	+ = 12 /2 8/ 23 6: 57 :0 0 A M	No effect [63,64]	=

	Engorgement (duration; blood meal size)	Smaller blood meal [33] Longer time to engorge [65]	+ ?		Smaller blood meal [36] No effect [27]	+ =		Longer, larger blood meal [34]	?
--	--	--	--------------------------	--	---	--------------------------	--	---	----------

479 Bold font indicates effects reported as statistically significant by the authors. The symbol in the right
480 column of each cell indicates the potential direction of the effect on virus transmission (“+” represents
481 an increase whereas “-” represents a decrease of virus transmission; “?” means that the direction of the
482 effect is undetermined and “=” means that there is no significant effect on virus transmission).

483



486 **Figure 1. Changes in mosquito behavioral traits that would enhance arbovirus transmission.** The
487 effects of arbovirus infection on mosquito behavior can occur in three functionally distinct cases: (1)
488 **Non infected mosquitoes.** Even if absent from the mosquito body, an arbovirus could indirectly
489 modify the behavior of mosquitoes through its effects on the human host, for example by making
490 human body odors more attractive [60]. (2) **Infected but non-infectious mosquitoes,** during the
491 extrinsic incubation period. Although these mosquitoes cannot transmit the arbovirus yet, flight, host-
492 seeking, and egg-laying behaviors could be manipulated by the virus to its advantage. While these
493 behaviors are primarily necessary for mosquito reproduction, they also increase the risk of death by
494 predation or host defense, and thus indirectly reduce the probability of subsequent virus transmission.
495 (3) **Infectious mosquitoes,** after the end of the extrinsic incubation period. At this stage, altered
496 behavior could directly enhance virus transmission, for example by increasing host-seeking activity,
497 the frequency of re-feeding attempts on multiple hosts, and the duration of probing or the amount of
498 saliva expectorated.