

1 **Title:** A new conceptual framework for host-microbe symbiosis

2 **Keywords:** symbiosis; transmission mode; partner fidelity; fitness effects

3 **Author:** Lily Khadempour

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5 **Correspondence:** lily.khadempour@rutgers.edu

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7 **Affiliation:** Department of Earth and Environmental Sciences, Rutgers University, Newark, Newark
8 New Jersey, USA

9 **Abstract:**

10 Host-microbe relationships are studied across biological disciplines, with unique but overlapping
11 conceptual frameworks arising from each of them. Without a unified framework that can be applied
12 across all host-microbe symbioses, we cannot do the interdisciplinary work necessary to understand
13 the underlying rules that govern them. Here I present a new conceptual framework for host-microbe
14 symbiosis, rooted in the original definition of symbiosis, across three axes: fitness effects, partner
15 fidelity and transmission mode. The three axes make a cube where any symbiotic relationship can be
16 placed. The position of a particular symbiosis is fluid and context-dependent, changing through
17 evolution and throughout the lifetime of an organism. The three axes make for a simple and
18 inclusive framework for symbiosis. We can use this framework to examine every known host-
19 microbe symbiosis, which will allow us to understand the rules and patterns that govern them.

20 **Lay Abstract:**

21 Researchers from many different fields study host-microbe symbiosis using different conceptual
22 frameworks and terminology. This makes interdisciplinary work challenging. This paper introduces a
23 new, unified way of thinking about host-microbe relationships that anyone in the field can use. It
24 organizes these relationships along three dimensions: how the relationship affects each partner's
25 fitness, how reliant each partner is on the relationship, and how the microbial symbiont is passed
26 from one host to another. Together, these three dimensions form a cube, and any host-microbe
27 symbiosis can be placed somewhere inside it. Importantly, a relationship's position in the cube is not
28 fixed. By using a shared framework, we can compare all known host-microbe relationships and start
29 to uncover the common rules that shape them all.

30

31 **Introduction:**

32 Symbiosis is a commonly used and contentiously argued term. It was first coined by De Bary
33 in 1879, often quoted as “the living together of unlike organisms” but in the English translation by
34 Oulhen et al. [1] “the living together of differently named organisms”. Regardless of the exact
35 wording, one important point is that, within this definition, symbiosis ranges from mutualistic
36 interactions where both partners benefit, to parasitic ones, where one partner benefits while the
37 other is harmed. It has long been accepted that these relationships exist on a spectrum rather than in
38 discrete categories [2–4]. This shift in thinking has allowed us to see that there are commonalities
39 and contrasts in how parasites, commensals and mutualists interact with their symbiotic partners [5].
40 It also allows us to think about how these relationships change: besides a few notable symbioses that
41 abruptly transition between categories, generally, symbioses shift along the fitness continuum with a
42 changing ecological context.

43 There is some debate around the bounds of symbiosis, particularly as it relates to the level of
44 intimacy necessary in a relationship to consider it a symbiosis. There are types of relationships that
45 are parasitism or mutualism that some scientists would not consider symbiosis, such as vampire bats
46 taking a blood meal of their host, or a bumble bee pollinating a flower in exchange for nectar,
47 because they are too ephemeral and the relationship is insufficiently intimate. It is argued in these
48 cases that the symbiotic partners do not spend enough of their life cycle with the other partner.
49 While I disagree with this perspective, that debate is one for the future. For this perspective, I focus
50 only on symbioses involving at least one microbial partner. Since microbes have rapid generation
51 times, one can assume that their time associated with a host involves multiple generations, so we can
52 consider all host-microbe relationships under symbiosis.

53 While it is commonly accepted that symbiotic relationships exist along a spectrum of fitness
54 effects, in this new framework I introduce two additional axes, allowing us to compare different

55 symbiotic relationships in multidimensional space. My hope is that this conceptual framework can
56 be used across disciplines to provide a unified definition and framework for host-microbe symbiosis.
57 By having a unified conceptual framework, I believe that we will be better equipped to understand
58 patterns in how symbioses occur and change in nature.

59 **Fitness effects**

60 As I briefly introduced above, the first axis of symbiosis to consider is that of fitness effects,
61 namely, how two organisms in a symbiotic relationship affect each other's fitness (Figure 1A). A
62 mutualistic relationship is one where both partners benefit. Some classic examples of mutualism
63 include aphids and *Buchnera* bacteria [6], bobtail squid and *Vibrio fischeri* [7], humans and *Bifidobacteria*
64 [8], algae and fungi in lichens [9] and legumes and nitrogen-fixing rhizobia [10].

65 Parasitism exists at the opposite end of the spectrum of fitness effects, where one partner
66 benefits at a fitness cost to the other partner. These relationships include those that are traditionally
67 grouped under the category of medical parasitology such as tape worms, lice, and *Giardia* [11], but
68 also any organism that is considered a pathogen, such as HIV [12] and pathogenic *Salmonella* in
69 humans [13] chytrid fungi that causes chytridiomycosis in amphibians [14], and *Escovopsis* in the
70 fungus gardens of leaf-cutter ants [15].

71 Between mutualism and parasitism lies commensalism, where one partner benefits and the
72 other experiences no fitness effects. Many examples of phoresy, where a smaller organism uses a
73 larger organism for dispersal, are considered commensal [16].

74 Every symbiotic relationship lies somewhere on the axis of fitness effects. However, its
75 position along the axis is not static, and can change through evolution and across ecological time
76 and space [17]. There are countless examples of instances of change across the fitness effects axis:
77 commensal microbes becoming opportunistic pathogens when the host's immune system is
78 compromised [18], the breakdown of the mutualism between corals and Symbiodiniaceae during

79 coral bleaching [19] and mutualistic endophytes evolving from latent pathogens [20]. Some
80 relationships are less context-dependent than others (Box 1). For example, the relationship between
81 fungus-growing ants and their cultivar is always a mutualism. This relationship has co-evolved for
82 tens of millions of years, and there is very little that can move the relationship from this position
83 [21]. But even within the mutualism space, one ant colony may serve as a more effective disperser
84 and so would provide greater fitness benefits to the fungus compared to a colony with less effective
85 dispersal. In this case, the relationship would not switch from being a mutualism to a parasitism, but
86 if we compared the two ant colonies across the axis, one would be further toward the extreme end
87 of mutualism than the other. This variation in symbiosis-related fitness effects between members of
88 a population, along with the variation in the environment in which the relationships exist, are the
89 fodder for evolutionary change across the axis.

90 **Partner fidelity**

91 Symbioses are often categorized as falling into one of two discrete levels of partner fidelity:
92 obligate or facultative. Classically, obligate is the case when at least one partner cannot survive
93 without the other, and facultative is when there may be fitness effects in a symbiosis, but the
94 partners can still survive without the other. I argue that the concept of a facultative symbiosis is just
95 a way to capture the inherent context-dependency of partner fidelity. We see this in the relationship
96 between legumes and their root symbiont rhizobia bacteria. This symbiosis is used as a classic
97 example of a facultative mutualism (Box 2). If a legume plant is grown in nitrogen-rich soil, the
98 number of root nodules that it produces are reduced because this function is not necessary for the
99 plant [22]. One could argue that *when nitrogen is present* in the soil, this relationship is facultative.
100 However, what happens if you grow this plant in soil with no nitrogen at all? I argue that this plant
101 can no longer survive without its mutualistic partner, and so the relationship would become obligate.

102 In this case, the partner fidelity depends largely on the availability of nitrogen in the local
103 environment.

104 As the legume/rhizobia example illustrates, instead of defining obligate and facultative as
105 discrete categories as we have always done, I think we should describe partner fidelity along a
106 context-dependent axis (Figure 1B). At one end we have obligate relationships where the partner
107 pair is always together, at the other end we have transient relationships where the pairing happens by
108 chance, and everything in the middle is facultative. What is a transient pairing? A host may acquire
109 any number of members of the microbiome that just end up living on or near the host for a time.
110 The relationship between host and a transient symbiont is ephemeral but presents an opportunity
111 for the symbiont and host to interact. For example, when any animal eats food, it will ingest some
112 microorganisms – if they do not die immediately they can turn out to be mutualists, commensals or
113 a parasites/pathogens. They may have no partner fidelity, and pass quickly from the system, but they
114 can still potentially have fitness effects on the host. Regardless of the fitness effects, these transient
115 relationships provide a novel niche for the symbiont, where the relationship may change to one with
116 more fidelity in the future.

117 Where a relationship lies on the spectrum from obligate to transient will depend on context,
118 and the position can change across ecological and evolutionary time scales. Like the fitness effects
119 axis, the flexibility of the position along the partner fidelity axis will vary for symbiotic partners.
120 There are some relationships in nature that we could classify as strictly obligate. *Chlamydia* and many
121 other intracellular parasites can only reproduce inside of host cells, and so context dependency is
122 low for the position of the relationship between *Chlamydia* and its host along this axis [23] .

123 Finally, the position along this axis will depend on which partner we are considering in the
124 relationship. In the case of *Chlamydia* (and other parasites) the relationship for the symbiont can be
125 obligate, but clearly, the host can and does survive without the symbiont. Therefore, the relationship

126 is obligate for the parasite but there is no fidelity from the perspective of the host, making this a
127 one-sided symbiotic relationship. Even for mutualisms, we can have one-sided symbioses. Corals
128 have an obligate relationship with Symbiodiniaceae but the algae can be free-living in the water
129 column. These asymmetrical relationships will be discussed further in the “three axes make a cube”
130 section.

131 **Transmission mode**

132 Transmission mode of a symbiont can vary widely, having important effects on the nature of
133 the relationship. Traditionally, transmission modes have been placed into discrete categories that
134 vary in their level of intimacy [24]: Vertical transmission is the most intimate and is where a
135 symbiont is transmitted from parent to offspring. Horizontal transmission occurs when a symbiont
136 is shared between two conspecifics, and is at an intermediate level of intimacy. And finally,
137 environmental transmission occurs when the symbiont comes from the environment. Within each
138 of the traditional transmission categories, there are already existing gradations, and they fall nicely on
139 a spectrum (Table 1).

140

141

142 Table 1. Categories and subcategories of transmission modes from most intimate (top) to least
 143 intimate (bottom).

Traditional Category	Subcategory	Real-world examples	Citations
Vertical Transmission	Intracellular endosymbiont passed to daughter cells	Prophage during binary fission; <i>Chlamydia</i> during mitosis	[25, 26]
	Non-intracellular endosymbiont transmitted through germ line	<i>Buchnera</i> housed in bacteriocyte and passed to egg; <i>Wolbachia</i> housed in ovaries and passed to egg	[27, 28]
	Symbiont transferred during birth, hatching, eclosion or germination	Infant acquiring symbionts during vaginal birth; Stinkbug gut symbiont transferred to egg capsule	[29, 30]
	Symbiont transferred from parent after birth, hatching, eclosion or germination	Infant acquiring symbionts during breast-feeding; fungus-growing ants acquiring protective actinobacteria from queen after eclosion	[29, 31]
Horizontal/ Vertical Transmission	Symbiont transferred from conspecific after birth, hatching, eclosion or germination	Infant acquiring symbionts during allomaternal care; fungus-growing ants acquiring protective actinobacteria from a nestmate after eclosion	[31, 32]
Horizontal Transmission	Direct contact between conspecific hosts	Sexual transmission of Hepatitis C virus	[33]
	No contact between conspecific hosts	Transmitting an infectious virus by coughing directly onto a conspecific	[34]
Horizontal/ Environmental Transmission	A symbiont remains in the environment in dormant form for an extended time	SARS-COV-2 remaining suspended in aerosols for many hours; acquisition of a long-dormant virus from thawing permafrost	[35, 36]
Environmental or Vector transmission	A symbiont has free-living generation(s) or associates with another host	<i>Vibrio fischeri</i> environmental acquisition by bobtail squid; Malaria parasite transmission by a mosquito vector	[37, 38]

144

145 I believe that our understanding of symbioses would be improved if we considered
 146 transmission modes along a spectrum, rather than discrete categories (Figure 1C). A classic example
 147 of strict vertical transmission is that of aphids and *Buchnera* bacteria, which the aphids house in
 148 specialized organs called bacteriocytes. The parent aphid uses a precise mechanism for ensuring the
 149 *Buchnera* is transmitted from its bacteriocyte into the embryo [27]. Vertical transmission can happen
 150 in a slightly less intimate way as well. For example, when a human gives birth through the vaginal

151 canal, the infant receives its initial microbiome from the parent. But the infant also receives some
152 members of its microbiome during the process of breastfeeding and by sharing intimate space with
153 its birthing parent [29]. At what point do we consider transmission of symbionts from parent to
154 offspring vertical transmission vs horizontal transmission from individuals who happen to be the
155 child's parents? Notably, if a child acquires symbionts from breastfeeding from a non-parent
156 individual, then this would be considered horizontal transmission even though the process of
157 acquisition is the same [32].

158 Similarly, the boundary between horizontal transmission and environmental transmission is
159 not clear. Let's consider the example of SARS-CoV-2. If someone infected with the virus infects
160 another person through close contact, this would be horizontal transmission. However, if someone
161 coughs the virus out in a room and it remains suspended in the air for several hours then another
162 person walks in and breathes it in, the transmission mode would exist somewhere between
163 horizontal and environmental transmission [39]. There are other symbionts, for example HIV,
164 *Chlamydia*, or the Hepatitis C virus that cannot be environmentally transmitted – they must be either
165 horizontally or vertically transmitted because they cannot persist outside of a host's tissues. Once
166 again, the position of a relationship along this axis is context-dependent but the level of context-
167 dependency varies from one relationship to another.

168 Importantly, some symbionts are transmitted by vectors or intermediate hosts. This may
169 seem to break the transmission mode axis. Instead, for the purposes of this conceptual framework, I
170 lump vector transmission with environmental transmission. Clearly, these phenomena are very
171 different, but if we focus on just the relationship between the two focal partners in a symbiosis, we
172 can use a separate cube for the relationship between the symbiont and vector. For our focal species,
173 whether a symbiont is transmitted through the environment or through a vector, it still requires
174 some kind of adaptation that allows for it to be separate from its symbiotic counterpart, either to

175 tolerate being in the environment, or to be successfully picked up by a vector. For the sake of
176 simplicity, environmentally acquired and vector transmitted symbionts can be grouped together on
177 the far end of the transmission mode spectrum from vertical transmission where the symbiont must
178 remain in constant contact with the host.

179 **Three axes make a cube**

180 Every symbiotic relationship can be placed somewhere in the three-dimensional space of a
181 cube created by the three axes (Figure 1D), occupying a cloud of probability space (Boxes 1-3). In
182 cases where there is not a lot of context-dependent flexibility, the cloud will be small, and where a
183 relationship is heavily context dependent, it will be large. The cloud of possible relationship space
184 will not necessarily be spherical. A relationship may be heavily context-dependent along one axis but
185 not the others. Through either ecological or evolutionary time, many symbiotic relationships may
186 transition gradually across the axis of fitness effects, from commensal to mutualistic or commensal
187 to parasitic, or even from mutualistic through commensal to parasitism. However, this is not the
188 case for all symbiotic relationships. Transitions between mutualism and parasitism can also happen
189 abruptly [40]. In these cases, the cloud of possibilities for a symbiotic pair would not pass through
190 commensalism. Instead, this relationship could be represented by two clouds of possibility at the
191 extremes of the axis of fitness effects. This should be the case for the other axes as well, and indeed
192 there can also be discrete clouds along the transmission mode axis. For example, dengue virus is
193 typically vector-transmitted but can also be vertically transmitted through umbilical cord blood,
194 however, it is never horizontally transmitted [41] (Box 3).

195 Earlier we encountered the problem of asymmetrical symbioses, particularly for the purpose
196 of partner fidelity (obligate for one partner but not for the other). To solve this problem, when we
197 make a cube for a symbiosis, we should note the focal species. The natural thing to do is to focus on
198 the host but even microbe-microbe symbioses can be put on this cube if a focal species is indicated.

199 As it is now, we can use this conceptual framework start to think about how symbioses will
200 fill it with hypothetical numbers based on the ecological and evolutionary patterns that we would
201 expect. However, important steps need to be taken to go from conceptual framework to theoretical
202 models. The next step is to create a standardized method for quantifying relationships across the
203 axes. This is not trivial, but some attempts have been made toward this goal [24, 42]. Once we have
204 this standardized method, then we can start populating the cube with all known symbiotic
205 relationships, across biological systems.

206 The cube can be used as a comparative tool to standardize how we describe symbioses. If we
207 populate the cube with every described symbiosis, then we may start to observe interesting patterns
208 or even rules. I expect that we will see more symbioses toward the edges of the cube, largely because
209 as scientists, we are drawn to striking phenotypes. But there may also be patterns that result from
210 the realities of biology. For example, obligate mutualists will tend to be vertically transmitted,
211 whereas parasites will be more likely to be horizontally or environmentally transmitted [42, 43]. One
212 rule that emerges from thinking in this holistic way is that environmentally transmitted obligate
213 symbionts must have a dormant phase. You can see this with viruses – all of them are obligate to
214 their hosts and all of them have a dormant particle phase when they are outside of their host cells.
215 This rule holds for parasites such as *Giardia* and *Chlamydia* as well.

216 I believe that by using this conceptual framework we will have a powerful tool to understand
217 patterns in symbiosis more broadly by examining which parts of the cube are more commonly
218 occupied. Specifically, we can ask questions about the tendencies and the mechanisms behind
219 transitions between locations on the cube. Are there parts of the cube that are harder to occupy and
220 why? Are there certain transitions that are more likely than others? Transmission mode, fitness and
221 partner fidelity are interrelated and, particularly as we consider evolutionary changes in symbiotic
222 relationships, we would expect there to be feedback between the different axes [43, 44]. Does

223 vertical transmission lead to mutualism, or does mutualism leads to vertical transmission? Given a
224 particular ecological context, will the future positioning of a symbiotic relationship be predictable? I
225 believe that by adopting this framework, where each factor of symbiosis is considered along three
226 interrelated axes, we will more easily be able to answer these types of questions.

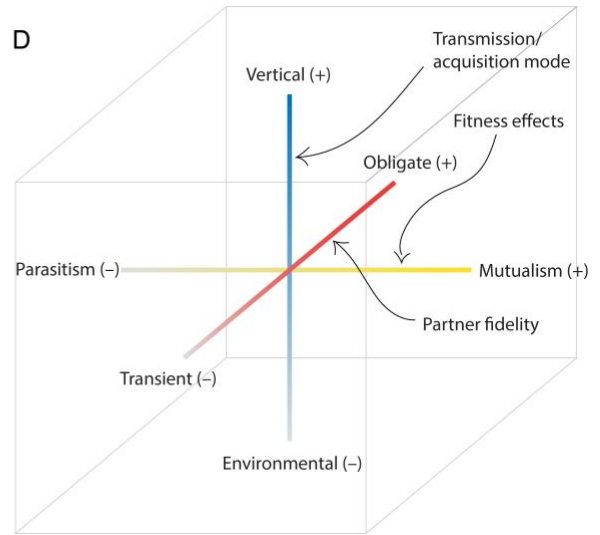
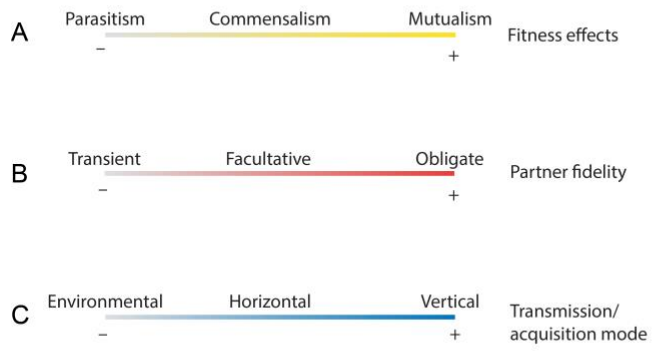
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233

234 **Figures**



235

Figure 1 The three axes of symbiosis separately (A, B and C) and as a cube (D).

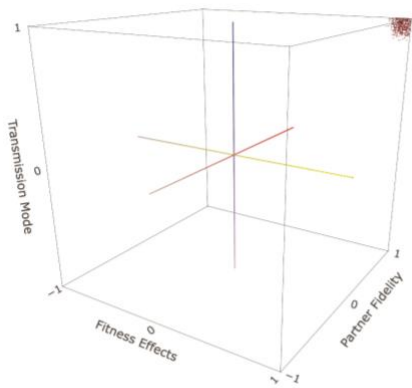
236

238 Box 1. Comparing two obligate, vertically-transmitted mutualisms

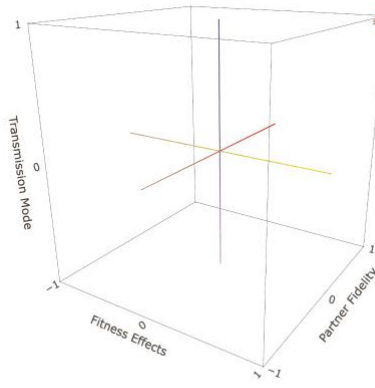
239 *Atta cephalotes*, like other fungus-growing ants, relies on a fungal cultivar to survive. The ants
240 cut leaves that they feed to the fungus, and the fungus produces gongylidia for the ants to eat for
241 most of their nutrition. Neither the fungus nor the ant can survive without the other. When an
242 unmated queen emerges from her mother's colony, she will carry a tuft of fungus in her infrabuccal
243 pocket and use that to start her own fungus garden. The relationship can be described as an obligate
244 mutualism with vertical transmission and would appear as a small cloud in the symbiosis cube [21] .
245 However, there is some variation and context-dependency in this system. There will be variation
246 between individuals in both populations of cultivar and ants that will make it so that along all three
247 axes, there will be some spread in the cube. Other contextual factors may vary as well. Other
248 community members in the fungus gardens can change the relationship between the ant and the
249 cultivar. For example, an *Escovopsis* parasite of the cultivar will cause harm to the fungus and will
250 increase the level of partner fidelity in the relationship because the cultivar will rely on the ants to
251 weed and groom their gardens. In the absence of the parasite, this relationship would be less
252 obligate. While the ants have a mechanism for vertical transmission, very rare horizontal
253 transmission events are possible, particularly during mating flights or if a colony moves into an
254 abandoned nest [45] . To visualize this relationship in the cube, we would see a small cloud in the
255 corner, near the extremes of all three axes.

256 The pea aphid *Acyrtosiphon pisum* and its bacterial endosymbiont *Buchnera aphidicola* also have
257 an obligate, vertically-transmitted mutualism [6, 27]. Across all three axes, this relationship is more
258 constrained and less context-dependent than the relationship between *A. cephalotes* and *L.*
259 *gongylophorus*. Therefore, it would appear as almost a dot on the symbiosis cube. Even for the
260 aphid/*Buchnera* relationship, there is variation between individuals in the populations of both

261 symbiotic partners, so it will not be an exact dot, but it will be a very tightly constrained cloud
262 compared to other more context-dependent symbioses.



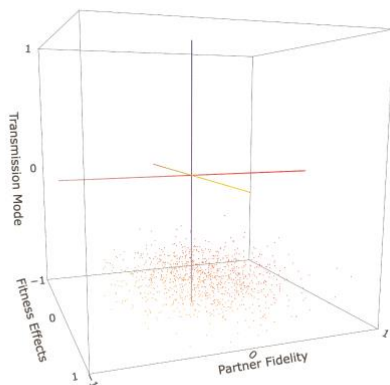
263 Leaf-cutter ant and *L. gongylophorus*



264 Aphid and *Buchnera*

265 **Box 2. A classic facultative symbiosis**

266 *Medicago truncatula* is a model legume plant that associates with *Sinorhizobium* spp. as well as
267 many other species of bacteria that provide nitrogen fixation to their host. Both the fitness effects
268 and partner fidelity are highly context dependent while the transmission mode is consistently
269 environmentally acquired. This type of relationship would create a flattened disk in the symbiosis
270 cube.



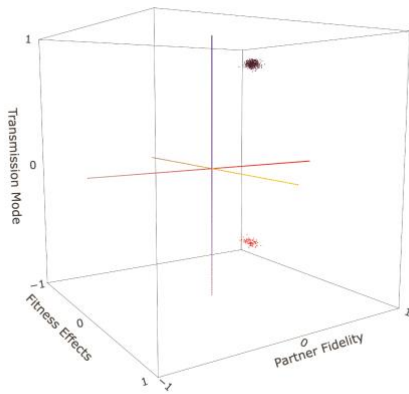
271 *Medicago* and *Sinorhizobium*

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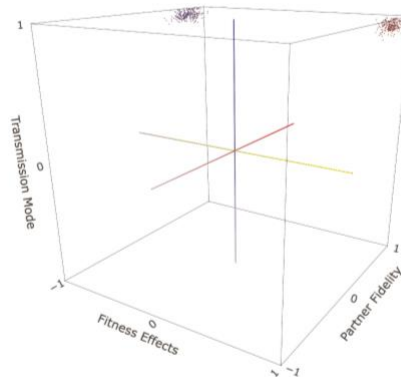
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273 **Box 3. Abrupt transitions across axes.**

274 While context-dependent transitions will often occur gradually across an axis, sometimes
275 there can be abrupt transitions. Here we have two examples of how these abrupt transitions can be
276 visualized. The first is of a plant endophytic fungus *Colletotrichum magna*, which through a single locus
277 mutation can go from being a pathogen to a mutualist, which acts as a defensive symbiont for its
278 host plant [40] . Next is the example of dengue fever, which is usually transmitted by a vector, but
279 can also be occasionally transmitted vertically through the placenta, but never through horizontal
280 transmission [41] .



281 Human and dengue virus



282 Host plant and *C. magna*

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