1 **An agent-based model of adaptation of holobionts with different microbial**
2 **symbiont transmission modes**

3 Running title: Simulation of holobiont adaptation to stress

4 **Siao Ye[1#*], Zhu Liu[2*] and Evan Siemann[1]**

5 [1]Department of Biosciences, Rice University, Houston, Texas, 77005, USA

6 [2] Department of Preventive Medicine, Keck School of Medicine, University of Southern

7 California, Los Angeles, 90007, USA

8 [#]Corresponding author: Department of Biosciences, Rice University, 6100 S. Main St., Houston,

9 Texas, 77005, USA; E-mail: thomasyesiao@gmail.com

10 *Siao Ye and Zhu Liu contributed equally to this work. Author order was determined both

11 alphabetically and in order of increasing seniority.

12 **KEYWORDS**

13 Endosymbiosis, holobiont, agent-based modeling, adaptation, stress

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ABSTRACT

The hologenome theory suggests that holobionts (host plus symbiont) with hosts that are only able to adapt slowly may be able to persist in deteriorating environmental conditions via rapid adaptation of their microbial symbionts. The effectiveness of such symbiont adaptation may vary depending on whether symbionts are passed directly to offspring (vertical transmission) or acquired from the environment (horizontal transmission). However, it has been suggested that holobionts with horizontal transmission cannot pass down their symbionts faithfully, preventing adaptation at the holobiont level because of host-symbiont disassociation between generations. Here we used an agent-based model to investigate whether holobionts with horizontal microbial symbiont transmission can adapt to increasing stress solely through symbiont adaptation and compared their adaptation to holobionts with vertical transmission. We found that holobionts with either transmission mode were able to adapt to increasing abiotic stress solely via symbiont adaptation. Moreover, those with horizontal transmission were more competitive than those with vertical transmission when hosts were able to selectively associate with the most suitable symbionts. However, those with horizontal transmission were less competitive than those with vertical transmission when symbiont establishment was random. Our results support the hologenome theory and demonstrate that holobionts with horizontal microbial symbiont transmission could adapt to increasing abiotic stress via their symbionts. We also showed that whether holobionts with horizontal or vertical symbiont transmission are favored in increasingly stressful conditions depends on the ability of hosts to recognize and foster microbial symbionts that confer stress tolerance.

36 IMPORTANCE

37 Symbiotic organisms such as reef building corals are sensitive to environmental perturbations

38 due to anthropogenic disturbances or climate change, and it is critical to understand whether they

39 are able to adapt to previously unfavorable conditions. To date, studies have focused on the

40 impacts of existing microbial symbiont variation on holobiont stress tolerance but here we use

41 agent-based models to explore holobiont adaptation via symbiont adaptation. We studied both

42 deterministic and stochastic processes in holobiont adaptation by investigating the following four

43 factors: holobiont transmission modes, a host's ability to recognize tolerance-conferring

44 symbionts, a symbiont's mutational variance, and rate of stress increase. Our simulation provides

45 a comprehensive understanding of holobiont adaptation under stress, which not only has

46 implications for future endangered symbiotic species management, but also provides fresh

47 insight into species evolution as proposed by the hologenome theory.

INTRODUCTION

Research on symbiotic organisms has suggested that symbiont variation can drive holobiont (host plus symbiont) phenotypic diversification, and influence holobiont fitness (1). In addition to host genetic changes, symbiont genetic changes can alter the holobiont phenotype and therefore impact holobiont adaptation (2). Zilber-Rosenberg and Rosenberg proposed that rapid holobiont adaptation could take place even when host adaptation is slow, because symbiont adaptation and composition shifts could be rapid (3). A diversity of potential symbiont partners are rich resources for holobionts, which might be critical for holobionts to adapt to increasing environmental stress such as global warming (4, 5).

Compared to hosts, symbionts have large population sizes and short generation times, and may adapt to ambient changes (6), yet it is unclear whether symbiont adaptation alone could drive holobiont adaptation. Most studies on holobionts focused on variation in symbiont composition rather than symbiont adaptation. For example, researchers found cnidarians associating with different symbiont strains differ in their thermal tolerances (5, 7), and thermal tolerance of aphids can be controlled by their symbiotic bacteria (8, 9). Such changes in holobiont phenotype are mainly based on existing symbiont diversity and can be observed within one generation. However, experiments testing holobiont adaptation via symbiont adaptation and composition shifts would be difficult because they require tracking of symbiont composition as well as holobiont phenotypes over a long time and so is less explored. Chakravarti et al. (10) pointed out that assisted evolution in symbionts can be applied to improve holobiont stress tolerance, and they were able to detect its impact on symbionts stress tolerance, but not on the holobiont stress tolerance. Some field observations also suggest holobiont adaptation can be achieved through symbiont adaptation. Rodriguez et al. (11) discovered the stress tolerance

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71   conferred by a symbiotic fungi to host plants is habitat dependent. Another study on corals also

72   revealed local adaptation existed in symbionts and leads to holobiont divergence in their thermal

73   tolerance, in which the symbiotic algae collected from warmer reefs maintained higher resistance

74   to heat compared to those from cooler habitats even after multiple asexual generations (12).

75   Nevertheless, it is hard to monitor symbiont adaptation in the field over evolutionary time scales

76   and track its impact on holobiont fitness. A simulation model would be appropriate to study

77   holobiont adaptation via symbiont adaptation (13, 14), which is lacking in holobiont research.

78   To investigate whether holobionts can adapt to stressful environments via symbiont

79   adaptation, it is critical to understand whether holobionts with either horizontal symbiont

80   transmission ("H" hereafter) or vertical symbiont transmission ("V" hereafter) are both able to

81   pass their adaptive symbionts to offspring, and which type is better at coping with stress. Some

82   researchers argue that passing down adaptive symbionts to offspring is unstable for holobionts

83   conducting horizontal transmission, which makes the hologenome theory less plausible (15, 16).

84   They suggest the symbiont community could be shaped simply by opportunity and

85   environmental filters, and the transmission fidelity is low (15, 16). In addition, opposite opinions

86   are given when discussing H's and V's adaptability. In vertical transmission, individuals inherit

87   symbionts directly from parents, which is similar to classical gene inheritance, so adaptive

88   symbionts from parents can be passed to offspring (17). But there are drawbacks associated with

89   this transmission mode; symbionts picked up by offspring might only be a subset of their

90   parents', so symbiont diversity could decrease dramatically through evolutionary time, and

91   become maladaptive once the ambient conditions change (18, 19). On the other hand, individuals

92   conducting horizontal transmission pick up a variety of symbionts in the neighborhood, so they

93   might respond to environmental changes faster by associating with stress tolerant symbionts (4,

94   20). However, the disassociation of symbionts and hosts between generations could lead to loss

95   of adaptive symbionts and colonization of virulent symbionts (21, 22). Most studies on microbe

96   transmission and adaptation in the past investigated how different transmission modes affect

97   virulence (17, 22–24), and explored the dynamics of virulence-transmission trade off instead of

98   considering them as a selection unit (25–27), because their systems emphasize antagonistic

99   rather than mutualistic relations between the host and the symbiont. Roughgarden (28) conducted

100   a pioneering simulation of holobiont evolution and found both vertically transmitted and

101   horizontally transmitted agents were able to evolve as a holobiont unit. But she used the number

102   of microbes to determine holobiont fitness in her model, and did not study the interactions

103   between the environment and holobionts. Because of the complex interactions among the host,

104   the symbiont, and the environment, combined with the different symbiont acquisition and

105   retention mechanisms in Hs and Vs, it is hard to predict whether Hs and Vs are able to adapt to

106   climate change, and which mode is more adaptive with increasing environmental stress.

107   Holobionts with different transmission modes could differ in their novel symbiont acquisition

108   and retention, which makes it hard to predict whether they are able to adapt to climate change,

109   and which modes is more adaptive.

110   Here we use agent-based modeling to explore how holobionts with different transmission

111   modes respond to increasing stress because such models are flexible and able to handle complex

112   problems (29, 30). Interestingly, such topics are better explored in cultural transmission, where

113   researchers explore how knowledge information (*e.g.*, language, knowledge, etc.) is transmitted

114   between generations under different environment regimes (31). In cultural transmission,

115   vertically inherited information (from parents) is more conservative than obliquely acquired

116   (from elder people excluding parents or ancestors) (32), which resembles vertically transmitted

117   symbionts and horizontally transmitted symbionts. Some studies reveal oblique transmission is

118   favored when the environment fluctuates, and is necessary in driving language evolution, while

119   vertical transmission is preferred when the environment is stable (33, 34). However, there could

120   be fundamental differences between cultural transmission and symbiont transmission. For

121   example, there is no increasing stress such as global warming in cultural transmission, so these

122   studies do not provide insights into whether both transmission modes allow holobiont adaption to

123   directional selection. In addition to transmission modes, we considered three other important

124   factors that might impact holobiont persistence in changing climate: 1) the host's ability to select

125   adaptive symbionts, 2) the rate at which stress increases, and 3) symbiont mutational variance.

126   We propose that the ability to select adaptive symbionts is a key factor when studying

127   holobiont adaptation, even though it is barely discussed, if at all, in previous research. Because

128   the amount of resources and space an organism can provide to symbionts is limited, holobiont

129   fitness is largely determined by the relative amount of adaptive symbionts within it (3, 35). So if

130   symbiont acquisition is completely random, then Hs will have no advantages over Vs even if Hs

131   have access to a larger symbiont pool from which they can choose partners, because the chances

132   of getting adaptive and maladaptive symbionts are the same. In addition, drift might have larger

133   impacts on Vs because the bottleneck effect is stronger in them due to the smaller effective

134   symbiont population size (18). On the contrary, if symbiont acquisition is not random but can be

135   determined by the host, then Hs might be more likely to associate with adaptive symbionts than

136   Vs, and drift impacts would be limited. Such symbiont selection by hosts is not uncommon and

137   has be found in both Hs and Vs, for example, solitary wasps block transmission of nonnative

138   symbionts to offspring, and sorghum selectively increases monoderm bacteria during drought to

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139   improve plant growth (36–38). Thus we tested the impacts of drift on Hs and Vs adaptation by

140   switching on and off the ability of holobionts to select for adaptive symbionts in our model.

141   Whether holobionts are able to adapt to the changing environment via symbiont

142   adaptation also depends on how fast stress increases and how fast symbionts adapt. Holobionts

143   such as corals are assumed to rely on the symbiont rather than the host to adapt to rising sea

144   temperature, because corals are living close to their thermal limits and the temperature is

145   increasing quickly (39, 40). Compared to the host, the symbionts are abundant with short

146   generations, so they are more likely the key to holobiont adaptation (3, 41). Because most

147   symbionts (*e.g.*, bacteria, algae, fungi) reproduce asexually (10, 42), we consider mutation the

148   major source of generating novel traits. Incorporation of mutation in our model makes the traits

149   continuous and dynamic instead of static and discrete, and generates intra-individual as well as

150   inter-individual symbiont variation. Such variation is necessary in understanding the complex

151   interactions between holobionts and the environment, and interactions among holobionts (43, 44).

152   Because population trait variance is sensitive to the magnitude of mutation (*i.e.*, mutational

153   variance) rather than mutation rate, it is important to know how mutational variance might affect

154   distribution of symbiont traits and thus holobiont adaptation (45, 46). Large mutational variance

155   may have greater impacts on horizontally transmitted holobionts, because once an extreme

156   symbiont strain arises, it can be transmitted throughout the population and may be hard to lose,

157   while it will be constrained in certain lineages in vertically transmitted holobionts. But large

158   mutational variance could also produce lineages that have accumulated extreme symbionts in Vs

159   when drift is strong. By varying the speed at which stress increases and mutational variance,

160   combined with controlling drift, we explored how the selection-mutation-drift balance interacted

161   with transmission modes.

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162   Our goal was to present a model to test whether holobiont adaptation can be driven by

163   associated symbiont changes, which involves symbiont mutation, acquisition, amplification and

164   transmission. The agent-based model we used simulated changes at the environment level, the

165   holobiont level, and the symbiont level, by varying four main parameters: transmission mode, the

166   ability to select adaptive symbionts, the rate of environmental change and the magnitude of

167   symbiont mutation. It involves interactions between the environment and the holobionts, among

168   the holobionts, and between the holobionts and the symbionts. We believe our model could

169   produce novel insights into holobiont adaptation.

170   **RESULTS**

171   **Test case**

172   We verified that when agents were selectively neutral in our basic model, the fixation was

173   random at a 1:1 V to H initial ratio (Fig. 1).

174   **Increasing temperature**

175   Regardless of whether transmission is random or optimal, both Vs and Hs were able to adapt to

176   increasing temperature when the rate of temperature increase was low and mutation variance was

177   high (Fig. 2).

178   Generally, holobionts persisted in high mutation variance and low temperature rate of

179   change conditions, but went extinct in low mutation variance and high temperature rate of

180   change conditions.

181   However, the transmission mode did affect the relative fitness of Hs and Vs (Fig. 3).

182   When transmission was completely random, Vs had higher fitness (Wilcoxon test, p=0.0002) and

183   longer time to extinction (Wilcoxon test, p=0.03) compared to Hs for the same parameter

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184   combination, and their gene values also had higher correlation with the temperature (Wilcoxon

185   test, p=0.0017). When transmission was optimal, Hs had higher fitness and longer time to

186   extinction, as well as higher correlation between gene values and temperature.

187   When both agent types were present at the same time, we were able to compare the ratio

188   of Hs and Vs (Fig. 4), which tells their relative competitiveness. In both random transmission

189   and optimal transmission models, Hs and Vs became extinct in large step/small mutation_sd

190   regions, and coexisted in small step/small mutation_sd regions. In the rest of regions, Vs

191   persisted in the random transmission model (Fig. 4a), and Hs persisted in the optimal

192   transmission model (Fig. 4b).

193   We also checked how virulence would affect the relative competitiveness of Hs and Vs

194   by reducing birthrate of Hs by 5% at mutation_sd=0.001 and step=0 in both models. This

195   resulted in Vs fixation in both models.

196   To explore how drift shapes symbiont distribution in the random transmission model, we

197   compared fitness variance between individuals and within individuals (Fig. 5). We found Vs had

198   larger variance among individuals (Wilcoxon test, p<0.001), but less variance within individuals

199   (Wilcoxon test, p<0.001). This means symbiont distributions in each vertical holobiont were

200   more homogenous than in each horizontal holobiont, but vertical holobionts were more

201   heterogeneous than horizontal holobionts across the holobiont fitness landscape.

202   **DISCUSSION**

203   We present here an agent-based model that investigates the adaptation ability of vertically

204   transmitted holobionts and horizontally transmitted holobionts to increasing stress through

205   symbiont adaptation. By introducing intraspecific trait variation and enabling evolution, we were

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206   able to study how selection-mutation-drift affected the population dynamics of holobionts with

207   different transmission modes. Our model clearly demonstrates that both Vs and Hs are able to

208   adapt to increasing environmental stress via symbiont adaptation, and their relative

209   competitiveness depends on whether symbiont transmission is random or not. As long as the

210   stress does not increase at a speed that will drive the holobionts to extinction rapidly, symbiont

211   adaptation alone could increase holobiont stress tolerance.

212   Our model suggests the answer to a long debated question: can horizontally transmitted

213   species be considered as a selection unit even though the symbiont disassociates from the host

214   during holobiont life history (16, 47)? We showed that although offspring do not necessarily

215   inherit symbionts from their parents directly, they could still acquire adapted symbionts. We

216   found stress tolerance conferred by symbionts improved over time as the mean symbiont gene

217   value increased along with increasing temperature. This was because holobionts whose symbiont

218   values better matched the temperature were more likely to survive, and they passed their adapted

219   symbionts to individuals in the next generation. In other words, because selection acts on the

220   population level and drives the symbiont to confer higher stress tolerance, individual holobionts

221   sampled from a symbiont pool that was adapting, and thus became adapted. This process looks

222   like a Lamarckian evolution because agents acquire stress tolerance by associating with stress-

223   resistant symbionts horizontally (48), but it is actually Darwinian evolution on the whole

224   symbiont population level since the symbiont gene frequency changes due to selection against

225   maladapted holobionts. Osmanovic *et al*. (49) found that selection on toxin tolerance of

226   vertically transmitted bacteria alleviated holobiont stress in a long-lasting toxic environment.

227   They suggested that horizontally transmitted symbionts can also be selected for and will confer

228   higher stress tolerance to hosts over time which is similar to our results here. Acquiring adaptive

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229   traits via associating with horizontally transmitted symbionts is commonly observed in nature. In

230   aphids, the secondary symbionts are considered to form a horizontal gene pool which facilitate

231   aphid adaptation (50). Redman *et al.* discovered fungal-free plants that received *Curvularia*

232   isolates (a type of fungal endophyte) from geothermal regions exhibited improved thermal

233   tolerance, which also suggests stress tolerance could be acquired horizontally (51). In addition,

234   research on corals with horizontally transmitted symbionts revealed surprisingly high fidelity in

235   symbionts across generations, indicating unknown mechanisms that maintain symbiont stability

236   in Hs (52). Thus we propose that symbiont-mediated holobiont adaptation is plausible in both Hs

237   and Vs.

238   Further comparison of Vs' and Hs' adaptation ability shows that whether holobionts are

239   able to identify and partner with suitable symbionts or not is critical in determining their relative

240   competitiveness. Researchers have opposite opinions regarding which transmission mode is

241   more advantageous because of following reasons: on one hand, Hs could exploit a broader range

242   of symbionts, and are likely to take up adaptive symbionts from the environment (21, 53), while

243   Vs can only inherit parents' symbionts, which may be maladaptive once the environment

244   changes (54, 55). On the other hand, adaptive symbionts could be passed down faithfully in

245   vertical transmission, but get lost during horizontal transmission (4, 15). Our results suggest both

246   arguments are partially correct, but are case dependent because symbiont acquisition could be

247   random or non-random, which has significant impacts on the outcome (56). When transmission

248   is random, Hs have no advantage even though the holobionts have access to a wider range of

249   symbionts, because the chances of taking up adaptive and maladaptive symbionts are equal; but

250   when they are able to recognize adaptive symbionts, they can pick up the fittest symbionts from

251   a larger pool compared to the limited choices Vs have, which greatly improves their performance.

252   In random transmission scenarios, drift is stronger in Vs and can drive symbiont fixation

253 in offspring lineages rapidly because they experience strong bottleneck effects given limited

254 symbiont options in parents (18, 57); in contrast, Hs offspring tend to maintain their parents'

255 optimum according to the law of large numbers, because they sample from a larger symbiont

256 pool (58). Our simulation of Hs and Vs without selection has shown that symbiont similarity is

257 higher within each V than within each H (*i.e.*, within host symbiont variation is larger in H

258 individuals than in V individuals), but lower among Vs than among Hs (*i.e.*, between-host

259 symbiont variation is lower among Hs than among Vs). This is consistent with studies on corals,

260 that found symbionts among vertically transmitted corals are more differentiated, and symbionts

261 among horizontally transmitted corals are less differentiated (52, 59). In psyllids, some hosts are

262 found to harbor only clonal symbiont lineages due to their vertical transmission mode, and the

263 low diversity within the holobiont suggests a strong bottleneck effect (60). Given enough time,

264 vertically transmitted symbionts could become so specific in host lineages that they will have

265 reduced genome sizes and cannot escape from hosts (61). The strategies of Vs and Hs are thus

266 different when facing environmental changes. Vs produce lineages that are distinctive in their

267 stress tolerance, while Hs produce individuals more likely to persist in all conditions. Because

268 maladaptive lineages that are dominated by maladaptive symbionts can be removed quickly from

269 V populations, while maladaptive symbionts can persist in H populations through coexisting

270 with adaptive symbionts, Vs are able to respond to environmental changes faster than Hs, and

271 thus adapt faster when symbiont transmission is random.

272   Moreover, extremely maladaptive symbionts could be transmitted across the whole H

273 population, while they are constrained in certain lineages in the V population, because of the

274 larger potential host population available for each symbiont in Hs (21). From this perspective,

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275   having maladaptive symbionts is similar to having parasites, which may provide additional

276   explanation for why higher virulence is often associated with horizontal transmission (24, 27).

277   Because maladaptive symbionts can be acquired by holobionts other than direct descendants,

278   they could persist in Hs just like infectious diseases, and decrease Hs' overall fitness. In contrast,

279   V individuals have a smaller sampling pool, which is determined by their parent, and offspring

280   that are quickly dominated by maladaptive strains due to drift can be selected against (57), so

281   overall fitness improves faster under selection. In short, once maladaptive symbionts arise in Hs,

282   they can be taken up and spread through the population and are unlikely to be eliminated, but in

283   Vs they can be trapped within lineages and be selected against, causing lower overall fitness in

284   Hs than Vs. In an extreme scenario when the stress increasing speed is zero (*i.e.*, under a

285   constant environment), although both populations could persist for the whole simulation, Vs had

286   slightly higher fitness especially when the mutational variance is large, where maladaptive

287   symbionts are likely to arise and spread in Hs. Given enough time, Vs should be able to

288   outcompete Hs (Fig. 5a). This resembles results of a study on virulence in pathogens, where

289   vertical transmitted pathogens were selected for to prevent host from acquiring more virulent

290   pathogens horizontally (27), and is consistent with study results of cultural transmission that

291   suggest Vs are favored under stable conditions (33, 62). Our simulation also support the

292   hypothesis that vertically transmitted holobionts such as corals may be more robust to climate

293   change (63), but conditional on them conducting random transmission.

294   When we incorporated optimal symbiont uptake into the model, surprisingly we had the

295   opposite result that Hs were better at tracking a changing environment. Such "recognition" of

296   suitable symbionts is not well explored in previous studies (28, 34), yet their impacts on the

297   outcome was very important in our simulation. We think such optimal transmission is equivalent

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298   to the positive transgenerational feedback proposed by Xue and Leibler (64). They suggested

299   phenotypes of parents are reinforced in offspring, so organisms could adapt to varying

300   environments. The positive transgenerational effect has been observed in some empirical studies,

301   which is probably caused by epigenetic processes (65, 66). Our previous experiment on green

302   hydra also showed that positive transgenerational effects could occur in holobionts simply via

303   transmission of acclimated symbionts. The model presented here is slightly different from that

304   described in Xue and Leibler's and examples mentioned above, because here we are focusing on

305   symbiont evolution rather than host evolution, and the phenotypes are distributed continuously

306   rather than discretely. Nevertheless, the acquired symbionts were derived from those hosted by

307   holobionts in the previous generations, which were more likely to be decedents of adaptive

308   symbionts than maladaptive symbionts in their ancestors. Because the temperature in our model

309   changes in one direction, the fittest new mutants are more likely to arise from the dominant,

310   fittest strain in parent, instead of from a less fit strain. Such horizontal transmission of stress-

311   tolerant symbionts has been observed in corals, which suggests holobiont adaptation can be

312   achieved through transmission of adaptive symbionts (67, 68). By having access to symbionts

313   released by holobionts other than their direct ancestors, Hs were able to selectively associate

314   with the fittest strain that arose in the population which is in contrast to Vs. The ability to pick up

315   the fittest symbionts, which is similar to copying from the most successful individuals in social

316   learning, increases Hs fitness and allows them to adapt faster than Vs (69).

317   There are two potential mechanisms that hosts are able employ to take in the fittest

318   symbionts: the first is via partner choice; the second is via passive up take. Mounting evidence

319   of partner choice is being shown for holobionts (70), for example, plants allocate more resources

320   to more beneficial partners and punish more parasitic partners (38, 71), so they could avoid

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321   cheaters and maximize their fitness during symbiosis. Bobtail squid are able to prevent

322   colonization of deficient luminescent bacteria *Vibrio fischeri* in their light organ, which could be

323   another case of partner choice (72). Massive coral bleaching could also be a case of hosts

324   ejecting maladaptive symbionts and establishing symbiosis with adaptive ones (40). Corals are

325   able to shift their symbiotic algae from sensitive strains to stress tolerant strains, so they can

326   become more robust to stress such as high temperature and UV (53, 73–75). Thus even coral

327   hosts that have long generation times and may not be able to adapt to warming temperatures

328   quickly enough still have a chance to withstand climate change (3). An interesting example of

329   wasps also shows that parents rather than the offspring may be able to choose symbionts to be

330   transmitted (37), as such transmission could be controlled on a cellular level (76). In addition,

331   even if the host is not able to identify the appropriate partner, they could form symbiosis with

332   symbionts that pass an environment filter. Because symbionts vary in their stress tolerance, a

333   strain that exhibits the highest fitness under certain conditions could outcompete other strains,

334   and then form symbiosis with hosts (77). Hosts taking up these adaptive symbionts would

335   acquire appropriate stress tolerance towards their environment (12). In insects, thermal stress

336   could easily wipe out their thermal sensitive symbiotic bacteria, which could reduce their

337   fecundity (8, 78). However, there are strains that could withstand such stress, and introducing

338   these strains into hosts can restore hosts' reproduction and enhance their thermal tolerance (8,

339   79). Symbionts that diverge in their niches are able to confer different traits and form association

340   with hosts in distinctive conditions, which facilitates holobiont diversification (9, 50, 80). These

341   examples provide solid evidence of optimal transmission of symbionts and suggest we should

342   take optimal symbiont transmission into consideration when studying holobiont persistence in

343   stressful environments.

344   Regardless of transmission mode and the ability to associate with optimal symbionts or

345   not, holobionts were more likely to persist under stress when their mutational variance was large

346   and stress increasing speed was low. It is not surprising that holobionts were able to persist

347   longer at low rates of increasing temperature, because other studies also suggest rapid increase in

348   stress could quickly wipe out the organisms (45, 81). However, Ayllón *et al.* (46) did not detect

349   significant impacts of mutational variance on adaptation, and they suggested it was because their

350   simulation ran for a limited number of generations (barely over 100 generations). In contrast, our

351   simulations lasted for 4000 generations. Moreover, whether holobionts went extinct or not

352   seemed to be confined by non-linear combinations of the mutational variance and temperature

353   increasing speed, which requires larger increase in mutational variance at high rates of

354   temperature increase than that at low rates of increase (Fig. 4).

355   Our models are the first exploring holobiont adaptation to stress, and demonstrate that

356   both Hs and Vs are able to adapt, even when symbionts and hosts are disassociated between

357   generations in Hs. Yet the model could be further expanded by incorporating more factors that

358   might affect the dynamics between Hs and Vs in different scenarios. For instance, conducting

359   horizontal transmission could be virulent, which might reduce Hs' overall fitness and their

360   competitiveness (27). In jellyfish, horizontal transmission favored symbionts shift to being

361   parasitic, which reduced the growth rate of jellyfish up to 50% (22). We have applied this only to

362   scenarios when there is no change in temperature, and found a 5% decrease in Hs' growth rate

363   could greatly reduce their relative frequency in both random transmission and optimal

364   transmission. This implies that under constant environments, vertical transmission with less

365   virulent symbionts would be favored (17, 33, 62, 82). But if we adjust the virulence parameter in

366   temperature increasing scenarios, we will be able to explore how virulence could alter holobiont

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367   population dynamics. Another factor worth exploring could be the tradeoff between thermal

368   tolerance and fitness cost. Studies on corals reveal that symbionts that confer higher thermal

369   tolerance may slow down the growth of corals (83, 84). Imposing such constraints may enhance

370   the authenticity of the model because holobionts will not be able to increase their thermal

371   tolerance infinitely. We can also investigate into how Hs and Vs react in other scenarios such as

372   random environments or fluctuating environments. We hope our models could provide a basic

373   framework for holobiont evolution and shed light on symbionts' roles from both ecological and

374   evolutionary perspectives.

375   **MATERIALS AND METHODS**

376   Our model was coded in python 3.0 with the agent-based modeling package "Mesa". Here we

377   present a model description following the ODD (Overview, Design Concepts, Details) protocol

378   by giving a summary of the overall model structure and processes (29). Detailed variables and

379   functions definitions can be found in Appendix A.

380   **Purpose**

381   The purpose of the model is to understand whether holobionts with vertical transmission

382   or horizontal transmission could adapt to changing environments solely depending on their

383   symbionts, and how drift and mutation would affect this process. The stress in this model is

384   called "temperature", which is a hypothetical stress without a real world metric because it could

385   also be called "salinity" or "UV intensity". Similarly, each timestep represents one generation

386   time, and has no corresponding real-world time length, because symbiotic species vary in their

387   generation time. To minimize the model complexity, holobionts (the agents) in our model are

388   autotrophic or heterotrophic with unlimited food and so do not need to compete for food, but

18

389   they have to compete for space, which is common in ecosystems such as coral reefs or for plants

390   (41, 85). Once a grid cell is occupied, it cannot be taken over by another agent unless the

391   previous occupier is dead.

392   **Scales and variables**

393   The model simulates adaptation of Vs and Hs under different temperature regimes, which

394   contains three hierarchical levels: 1) environment, 2) holobiont, 3) symbiont. The object of the

395   environment level is temperature. The temperature is a standardized hypothetical parameter

396   without units because we are not studying a specific species and a specific stress tolerance, but

397   rather to verify if holobionts can evolve as a unit. Each time step represents one generation, and

398   agents could die or give birth to one offspring. The objects of the second level are the two types

399   of holobionts, Vs and Hs, which only differ in their transmission modes. To track the adaptability

400   of Vs and Hs, agents reproduce asexually and do not switch their transmission modes across

401   generations. This allowed us to better track symbiont compositions of the two agent types over

402   time, and compare fitness of Vs and Hs. The object of the third level, symbiont, is the only factor

403   that determines holobionts' fitness. Because our goal is to test whether both Vs and Hs can adapt

404   via symbiont adaptation, and many holobionts' stress tolerance is largely determined by

405   symbiont stress tolerance (40, 86–88), we assume no host impacts on holobiont's stress tolerance

406   in this model. Each agent contains a fixed number of symbionts, and each symbiont is assigned

407   a randomly generated gene value from which the host fitness will be calculated.

408   **Scheduling**

409   The model is executed in following steps:

410   (1). Model Initialization

19

411   Initially 2000 agents are generated and randomly distributed on a 50*50 size grid. In

412   cases where both Hs and Vs are presented, each agent has 50% chance to be H and 50% chance

413   to be V. Each agent contains 50 symbionts, whose value is drawn from a normal distribution

414   with mean equal to 0.5 and variance equal to mutational variance, truncated at two standard

415   deviations (46, 89). Initial temperature is set at 0.5 as well.

416   (2). Holobiont reproduction

417   At every time step, each agent has a chance to reproduce based on its fitness, which is the

418   mean of the fitness score calculated for its symbionts, assuming all the symbionts within the host

419   will affect the holobiont's fitness. We used a symmetric beta distribution to calculate the fitness

420   score (90), which reaches the maximum when the symbiont's gene value matches current

421   temperature (Fig.6), and reaches a minimum when the mismatch is equal or greater than one, in

422   other words, the current temperature could be either too hot or too cold for the symbiont. A

423   random number is generated and compared to the holobiont's fitness value to decide whether it

424   will reproduce or not. Because conducting horizontal transmission might suppress holobiont

425   reproduction, we introduce a cost parameter "l" that could reduce H's reproduction probability

426   (82).

427   (3). Symbiont transmission

428   In the default model, drift is introduced by enabling random symbiont transmission in Hs

429   and Vs. V offspring sample symbionts from their parents randomly with replacement, so their

430   symbionts could be a subset of those in their parents. H offspring sample symbionts from other

431   agents around them once they are produced and dispersed (17). This transmission process is

432   equivalent to picking up symbionts from a common pool constitute of symbionts released from

433   agents (28).

434   In the optimal transmission model, drift is suppressed by enabling agents to pick the

435   fittest symbionts from parents. As in cases where certain symbiont strains is dominant while the

436   rest strains are marginal, here offspring choose the fittest strain from the parent (in V) or from

437   neighboring agents (in H) to make up the symbiont population, and then randomly sample with

438   replacement as in the default model to fill the rest of its symbiont population.

439   (4). Symbiont mutation

440   During holobiont reproduction, the symbiont may mutate by chance once it is acquired by

441   the offspring. The mutation probability of each symbiont strain within the holobiont is set at 0.01

442   (91), given that symbiont generation time is shorter than holobiont generation time while the

443   abundance is large (92, 93). The mutated symbiont gene value is drawn from a normal

444   distribution with mean centered on the original symbiont gene value, variance controlled by the

445   mutational variance, truncated at two standard deviation from the mean (46). Since we are

446   interested in whether symbiont gene changes could drive holobiont adaptation, we assume

447   heritability equals one and there is no environmental variance in our model.

448   (5). Holobiont dispersal

449   We use a stepwise function to control the position of a newborn holobiont. Because the

450   further away from the parent the less likely the offspring would occur (94), we set equally high

451   probability within certain distance for the new holobiont to occupy the gird, and equally low

452   probability beyond certain distance for the holobiont to occur.

21

453   (6). Selection

454   Agent fitness is calculated as described above, except for the decision whether the agent

455   will die or not.

456   Detailed parameters are documented in appendix A, and each parameter combination was

457   run for 4000 time steps and at least 20 iterations.

458   **Design Concepts**

459   Emergence: Population dynamics emerge from the reproduction, selection and

460   competition of the two types of agents, embedded with symbiont drift and mutation. Adaptation

461   is driven by interactions between each agent and the environment.

462   Sensing: Agents can perceive the temperature and know their transmission modes. In the

463   optimal transmission model, agents are able to pick the fittest symbionts from their ancestors.

464   Interaction: Competition for space (empty cells) is modeled explicitly, based on a first

465   come first serve rule. Reproduction and selection is simulated based on the beta functions (90)

466   which depict the relation between fitness and temperature stress.

467   Stochasticity: At each process, the order of agents to execute the code is random. All

468   behavioral parameters are generated based on empirical probability distributions. That means the

469   reproduction, dispersal, and selection of agents are not deterministic, and symbiont values after

470   mutation and inheritance in each agent are random. This introduces stochasticity on both

471   symbiont and holobiont levels, and the final results are emergent from interactions between

472   individuals and the environment.

22

473   Observation: For model analysis, population-level data were recorded, i.e., population

474   size over time, relative ratio of the two agent types, mean holobiont optimal temperature, and

475   time to extinction, etc. (95).

476   **Simulation scenarios**

477   (1) Test case

478   To test and validate the model, we constructed a basic model that both agent types have

479   equal fixed reproduction and survival probability, so they are selectively neutral and their fitness

480   does not depend on the environment. This could be viewed as two genotypes in a finite

481   population, whose fixation should be random. According to Kimura & Ohta (57), when the

482   initial relative ratio is 0.5, either H or V will fix at 50% chance, and if the initial relative ratio

483   moves away from 0.5, one or the other will be more likely to fix within dramatically less time.

484   (2) Increasing temperature

485   The temperature was projected to increase at different speed infinitely, which means

486   holobionts would only be able to persist by having novel mutations conferring thermal tolerance

487   higher than that of existing symbionts. This model tested whether both Vs and Hs could keep up

488   with increasing stress, and explored how symbiont mutational variance might affect this process

489   given different increasing speed. For both random transmission model and optimal transmission

490   model, Hs and Vs were simulated in coexistence and in separation.

491   **Statistical analysis**

492   We used paired Wilcoxon rank tests to compare the fitness between Hs and Vs across all

493   the parameter combinations. We summed up the mean holobiont fitness at each time step of

494 every iteration for a given parameter combination and performed a log transformation. This

495 provides us information that includes both the fitness at each time step as well as the extinction

496 time. We used the same test for Hs and Vs extinction time, correlation between holobiont gene

497 values and the temperature, fitness score variance within holobionts, and fitness score variance

498 between holobionts.

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737   ACKNOWLEDGEMENTS

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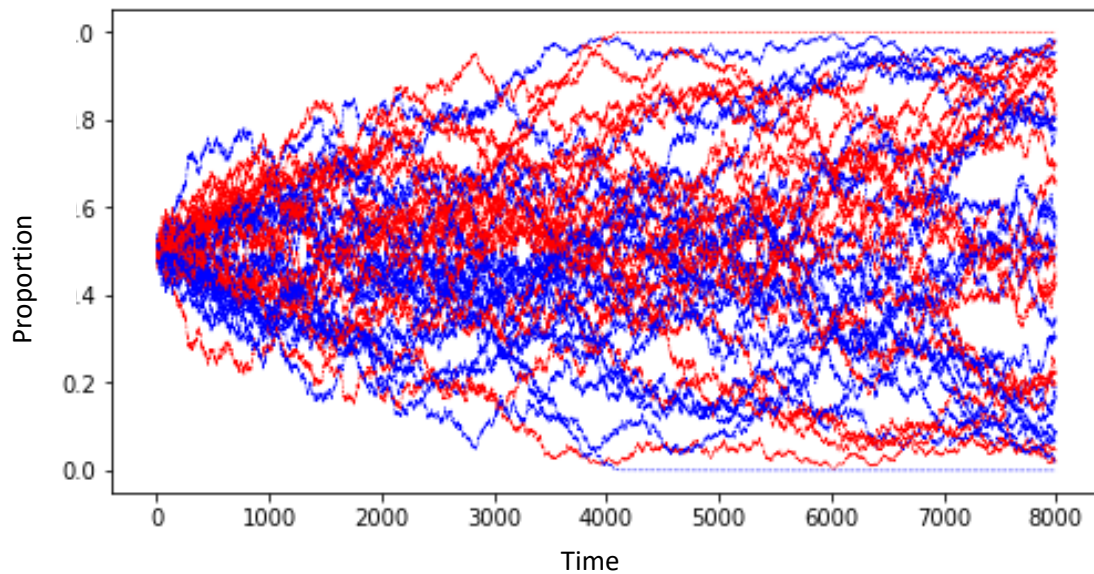
743   Figure 1: Fixation of holobiont transmission types under selectively neutral conditions and

744   equal starting abundances of vertical and horizontal transmission types. Blue lines indicate

745   the proportion of holobionts that have vertical transmission (V) and red lines indicate the

746   proportion that have horizontal transmission (H). Each simulation is represented by a

747   single blue line and single red line.

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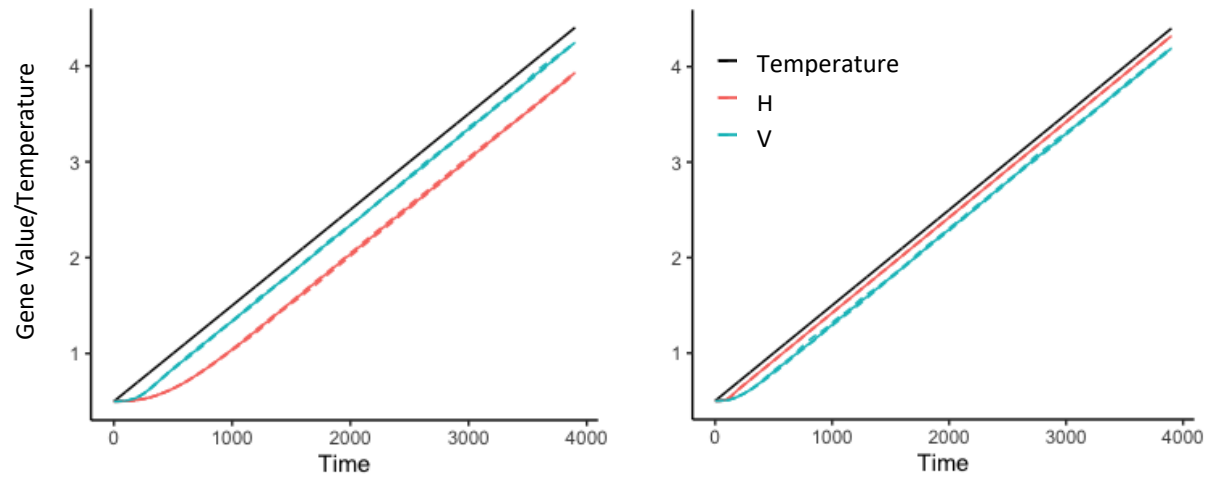
Figure 2: Average symbiont gene values (optimal temperature value) of horizontal (H) and

vertical (V) transmission mode holobionts in (a) the random transmission model with

temperature rate of change per time step=0.001 and magnitude of mutation with standard

deviation=0.1, (b) the optimal transmission model at step=0.001, mutation_sd=0.01.
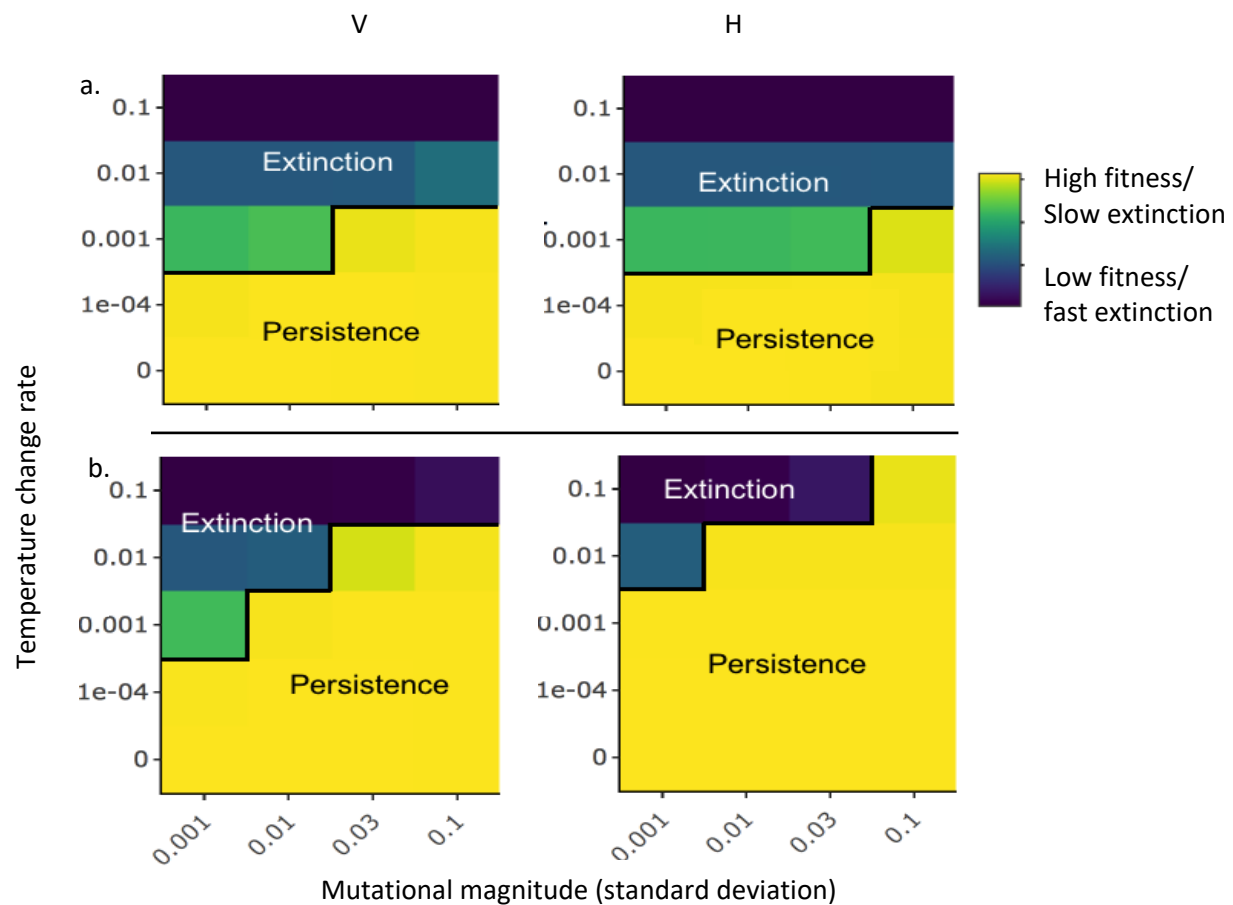
Figure 3: The dependence of fitness and time to extinction of vertical (V) or horizontal (H) transmission holobionts in separate simulations on rate of temperature change (magnitude of change per time step) and mutation magnitude (standard deviation) with (a) the random transmission model, (b) optimal transmission model. Rapid temperature change (large change per time step) and small magnitude mutations (low mutation variance) are likely to lower holobiont fitness and lead to extinction. The scenarios with persistence are those with high average fitness and those with low fitness have rapid extinction.

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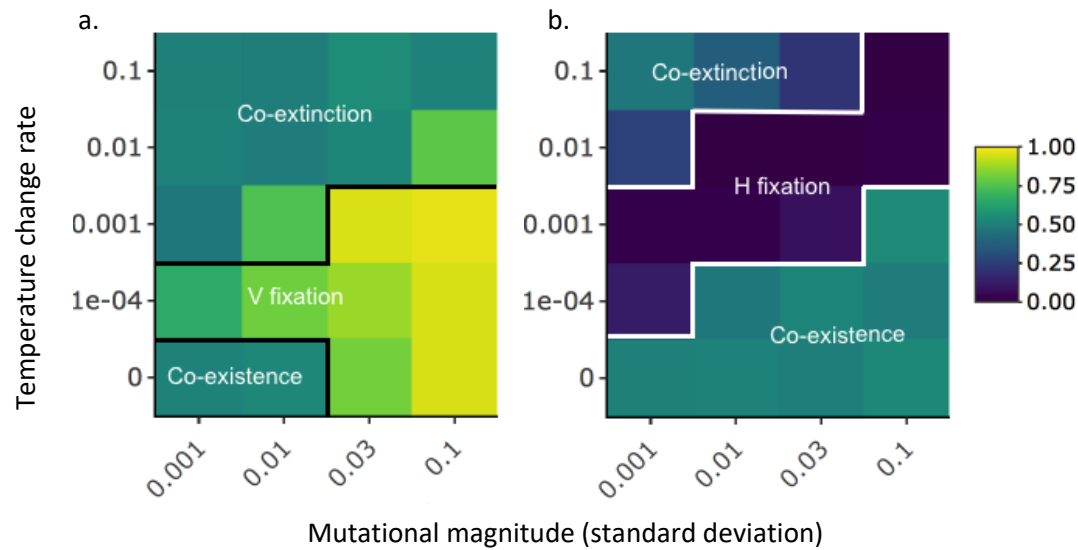
Figure 4. The dependence of average proportion of vertical transmission holobionts in simulations that include both vertical and horizontal transmission types on rate of temperature change (magnitude of change per time step) and mutation magnitude (standard deviation) with (a) random transmission, (b) optimal transmission. Brighter colors indicate conditions in which vertical transmission types reached fixation more quickly (and horizontal types became extinct) and darker colors indicate conditions in which vertical transmission types became extinct faster (and horizontal types reached fixation).
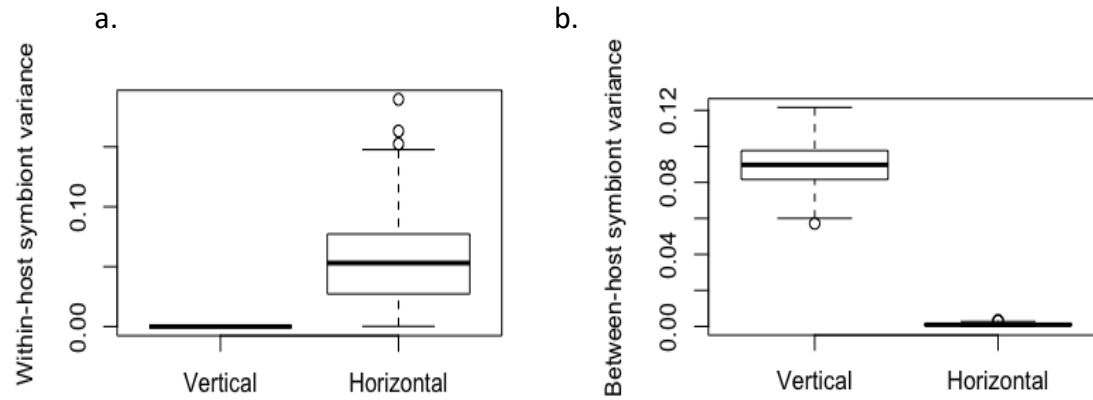
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784   Figure 5: Variance of symbiont fitness scores (a) within hosts (average of the variance of scores

785   for symbionts within single hosts), (b) between hosts (the variance of the average scores of hosts).

786   Each point is the variance in a single random transmission simulation with only vertical or

787   horizontal transmission types after 1000 time steps.

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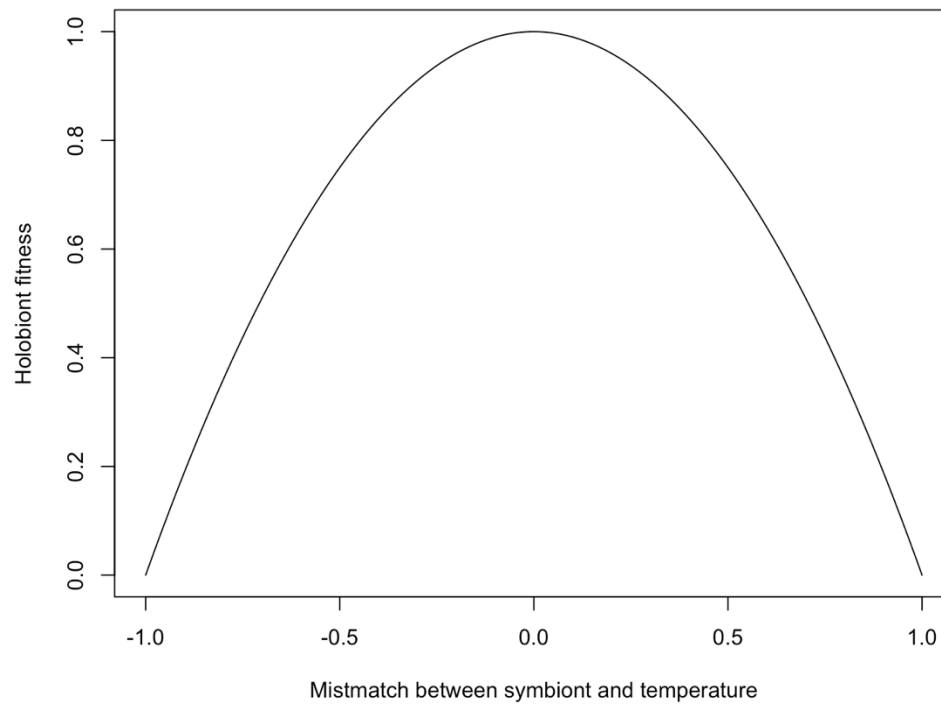
Figure 6: Fitness curve. The fitness impact of each symbiont on the holobiont (fitness score) was

calculated based the difference between the symbiont gene value (optimal temperature) and the

current temperature. When the symbiont gene value matched the current temperature, the fitness

score was set equal to 1 and it declined (beta function) as the gene value and temperature were

more different. A positive mismatch value indicates the environment is colder than the optimal

value for the symbiont and a negative value indicates the environment is colder than the optimal

value.