Dirty Transmission: Increased Mutations During Horizontal Transmission Can Select for Increased Levels of Mutualism in Endosymbionts

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Abstract

A mutualistic symbiosis occurs when organisms of different species cooperate closely for a net benefit over time. Mutualistic relationships are important for human health, food production, and ecosystem maintenance. However, they can evolve to parasitism or breakdown all together and the conditions that maintain and influence them are not completely understood. Vertical and horizontal transmission of mutualistic endosymbionts are two factors that can influence the evolution of mutualism. Using the artificial life system, Symbulation, we studied the effects of different rates of mutation during horizontal transmission on mutualistic symbiosis at different levels of vertical transmission. We propose and provide evidence for the "Dirty Transmission Hypothesis", which states that higher rates of mutation during horizontal transmission can select for increased mutualism to avoid deleterious mutation accumulation.

Introduction

Mutualistic endosymbiosis — close and long term cooperation between species where one organism lives inside of another – is a widespread and well-established phenomena in the biological world (de Vries and Archibald, 2017; Archibald, 2015; Zachar and Boza, 2020; Lazcano and Peretó, 2017; Johnson et al., 2021). These mutualistic relationships impact humans in a number of ways, including human health, food production, and the maintenance of ecosystems around the world (Toby Kiers et al., 2010). Common examples of mutualistic endosymbiosis include the human gut microbiome as well as the root-nodule bacteria of legumes (Drew et al., 2021; Trivedi et al., 2020).

While mutualism can be rewarding, there are risks to engaging in it. There is always a chance that one partner in a mutualistic relationship will cheat, increasing its own fitness to the detriment of the other and potentially shifting into parasitism or causing the mutualism to breakdown completely (Jones et al., 2015; Moran et al., 2008). Further, mutualistic endosymbiosis is a particularly tight relationship, changing the environment of one of the species completely and often leading to host species' dependence on its endosymbionts (O'Malley, 2015). Thus, there is the question of under what conditions mutualistic endosymbioses emerge and what factors influence them the most. Previous research has shown that mutualistic endosymbioses can be influenced by the rate of vertical transmission (Vostinar and Ofria, 2019; Bruijning et al., 2021; Shapiro and Turner, 2014). Vertical transmission is when a host reproduces and its offspring are infected by its symbiont (Fine, 1975). There is also a second mode of symbiont transmission, horizontal transmission. Horizontal transmission is transmission that is not linked to reproduction. (Ewald, 1987).

Symbionts can incur mutations in their genomes, which could in turn impact their fitness and relationships with their hosts (Drake, 1991; Drake et al., 1998; Drake and Holland, 1999; Sanjuán et al., 2010). It is possible that symbionts may accumulate more mutations during horizontal transmission because they must leave their hosts and expose themselves to the environment, potentially leaving them open to more damage to their genome. In addition, some symbionts may experience further decreased mutation rates during vertical transmission due to host repair mechanisms. For example, temperate bacteriophage specifically can have the benefit of host genetic repair mechanisms while lysogenized, potentially decreasing their realized mutation rate when vertically transmitted compared to when horizontally transmitted through lysis (Duffy et al., 2008).

In such a system with a higher mutation rate during horizontal transmission and an intermediate chance of vertical transmission, a symbiont that has evolved to rely on horizontal transmission could have more offspring than a symbiont evolved to rely on vertical transmission. However, if most of the offspring transmitted horizontally acquire deleterious mutations, the symbiont with the vertical transmission strategy could actually have higher fitness. Symbionts with a vertical transmission strategy should then also be under selection to be more mutualistic to improve their host's fitness and therefore their own.

To our knowledge, there is no previous research on how a higher mutation rate during horizontal transmission might impact the evolution of mutualistic relationships. Controlling and detecting the mutation rates during different transmission modes is challenging if not impossible in most biological systems (Peck and Lauring, 2018). Therefore, to test this hypothesis, we used an artificial life system called Symbulation, where a population of hosts and endosymbionts are able to co-evolve between antagonistic and mutualistic behavior (Vostinar, 2021; Vostinar et al., 2021). Using this system, we were able to test how horizontal transmission-associated mutation impacts mutualistic relationships. We determined that at intermediate vertical transmission rates, a higher relative mutation rate during horizontal transmission selects for a stable mutualism where otherwise parasitism dominates. These results support the "Dirty Transmission Hypothesis" and thereby provide an additional mechanism that could tip the balance towards mutualism when an endosymbiotic relationship is first evolving.

Methods

For this investigation, we used the Symbulation platform (Vostinar, 2021) to enable endosymbiotic relationships that could evolve between parasitism and mutualism. The evolutionary agent-based simulation consists of hosts and endosymbionts that each have their own genome consisting of one value, the interaction value. As shown in Fig. 1, this value dictates the amount of cooperation or antagonism that that organism will engage in and ranges from -1 to 1. We expanded Symbulation, as shown in Fig. 2, such that endosymbionts have an additional trait, their efficiency value, which determines how effective they are at processing resources into a usable form for themselves, and is an abstraction of the many traits that can contribute to endosymbiont fitness other than how they interact with their host.

At each time step, every host receives 100 resources that can be used for reproduction, defense, or distribution to its endosymbiont (if it has one). Each host can have up to one endosymbiont, restricting multiplicity of infection to 1 or less. Endosymbionts can receive or steal resources from hosts, as well as donate resources back to hosts. These behaviors are dependent on host and symbiont interaction values[1].

Interaction Value

Interaction values below 0 indicate antagonism between partners. An endosymbiont with a negative interaction value will attempt to steal that proportion of resources from its host, while a host with a negative value will invest that proportion of its resources into defense. When resources are used for defense they are no longer available to be used for reproduction or transmission. The amount of resources stolen from the host is the difference between the endosymbiont and host interaction values, assuming the endosymbiont's interaction value is more negative. If the host value is positive and the symbiont value is negative, the host donates

[1]This trait was referred to as *resource behavior value* in previous work. Here we use the term *interaction value*.

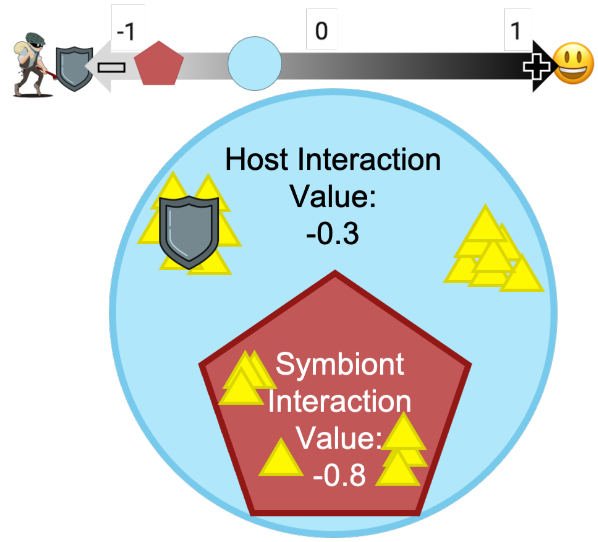

Figure 1: **Overview of host and symbiont interaction.** Each host can have up to one symbiont. The behavior of both organisms is determined by their interaction value. A negative interaction value indicates antagonistic behavior whereas a positive interaction value indicates mutualistic behavior.

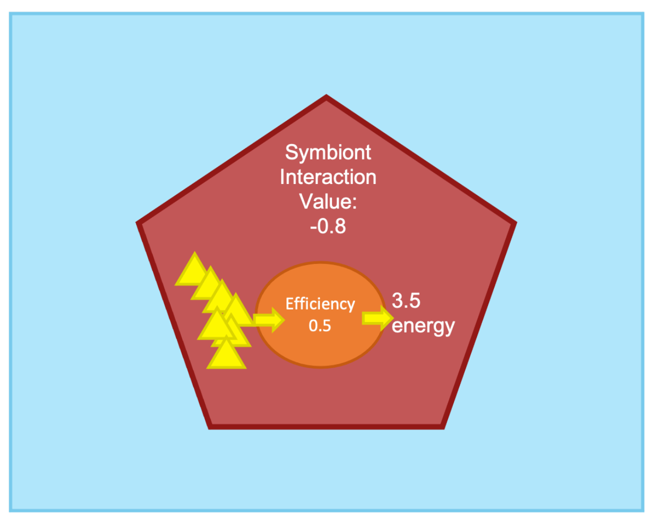

Figure 2: **Overview of the efficiency trait of symbionts.** Each symbiont has an efficiency trait, which determines how effective they are at using resources regardless of how they receive those resources. A lower efficiency value means a symbiont is able to glean less energy (for reproduction) from the resources it has.

Table 1: Results for host and symbiont interaction values

Host IV	Symbiont IV	Result
$X > 0$	$Y > 0$	Host donates proportion X to symbiont, symbiont donates proportion Y back, which is multiplied by 2
$X < 0$	$Y < 0$	Host invests proportion X in defense, symbiont steals proportion X - Y, host gets what remains
$X > 0$	$Y < 0$	Host donates proportion X to symbiont, symbionts steals additional proportion Y, host gets what remains
$X < 0$	$Y > 0$	Host invests X in defense, symbiont has no resources to donate, host gets remaining resources

the appropriate proportion of resources and the symbiont steals a further proportion of resources from those that the host attempted to keep for its own reproduction. Conversely, if the symbiont value is positive and the host value is negative, the host invests in defense and the symbiont receives no resources. All possible impacts of interaction value combinations are shown in Table 1, adapted from (Vostinar and Ofria, 2019).

Interaction values above 0 indicate cooperation between partners. A host with a positive interaction value with donate that proportion of its resources to its endosymbiont. An endosymbiont with a positive value will donate that proportion of resources back to the host, multiplied by a synergy factor of 2. The synergy factor is meant to represent the benefit of participating in mutualism and sharing resources. Previous research has evaluated the use of division of labor across multiple resources instead of an artificial synergy factor and found similar results (Vostinar and Ofria, 2019).

Reproduction

Both host and endosymbiont interaction values and the endosymbiont's efficiency value are subject to mutation upon reproduction and transmission. A host can reproduce after it accumulates 1000 resources, at which point its offspring is placed at a random location in the world, killing any organisms already existing in that space. The offspring's interaction value has a chance of mutating based on the mutation rate. If the interaction value mutates, a random number is generated from a normal distribution with a mean of 0 and a standard deviation of 0.002 and the value is changed by that amount. In all experiments in this work, the host mutation rate is fixed at a 10% chance.

Transmission of symbionts can occur under two circumstances. First, when a host reproduces, its symbiont has a set chance between 0 and 100% of vertically transmitting a symbiont offspring to the host offspring. Second, a symbiont can horizontally transmit its offspring after accumulating 100 resources. A random host is selected for the symbiont offspring to infect; if that host is already infected with a symbiont, the offspring will die. Because both host and symbiont offspring (through horizontal transmission) are placed in random locations in the world, the environment is spatially unstructured and therefore akin to a well-mixed liquid environment. Hosts can only have one symbiont, and that symbiont cannot be removed.

Symbiont Mutation Rates

Mutations to the interaction and efficiency values of the symbiont can occur during both vertical and horizontal transmission. For this work, we controlled the mutation rates during horizontal and vertical transmission separately for both interaction and efficiency value. For the interaction value, this means that when transmission occurs, there is a chance for mutation of the interaction value dependent on what type of transmission is occurring. We held the vertical transmission-associated mutation rate constant at 10% for both traits. We then tested the degree of mutualism, measured by the interaction value, when changing the *horizontal transmission-associated mutation rate (HTMR)* for 1) both the efficiency and interaction value, 2) only the interaction value, and 3) only the efficiency value across the full spectrum of vertical transmission rates.

Experimental Settings

Experiments had 30 replicates, ran for 10,000 time steps, and had a population limit of 10,000 hosts. The environment was a 2D well-mixed torus, and experiments began with a full population of hosts and symbionts with randomly generated interaction values.

Symbulation is built on the Empirical library (Ofria et al., 2020) and all code and scripts for this work are available under the MIT license at (redacted for double blind review).

Statistical Analysis

All plots were created in RStudio (R Core Team, 2020) using the ggplot2 package (Wickham, 2016) and the Viridis package (Garnier et al., 2021). For all significance tests, we conducted Wilcoxon rank-sum tests. We applied a Bonferroni correction for multiple comparisons to all p-values.

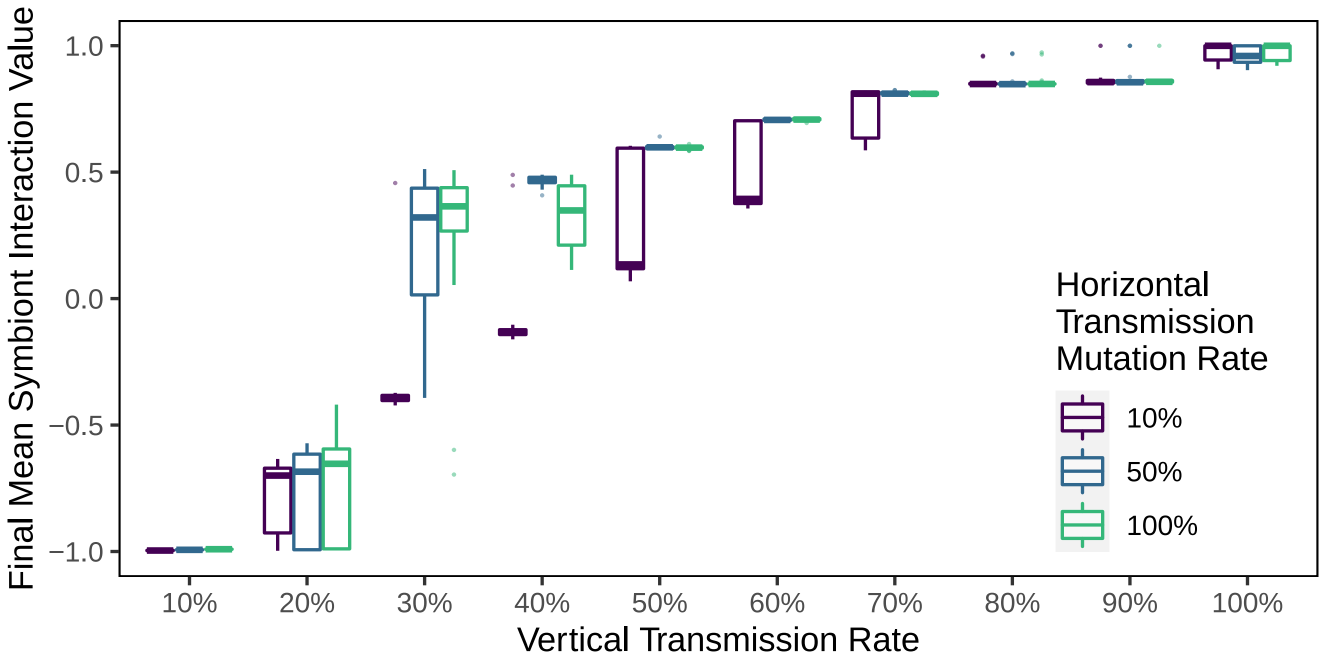

Figure 3: **Mean symbiont interaction value across vertical transmission rates when the rate of mutation during horizontal transmission was increased.** The mutation rate during vertical transmission and host reproduction was held at 10%. The difference between HTMR 10% and 50% is significant at vertical transmission rates of 10, 30, 40, 50, and 60% ($p < 0.005$ for all comparisons). The difference between HTMR 50% and 100% is not significant at any vertical transmission rates after correction for multiple comparisons.

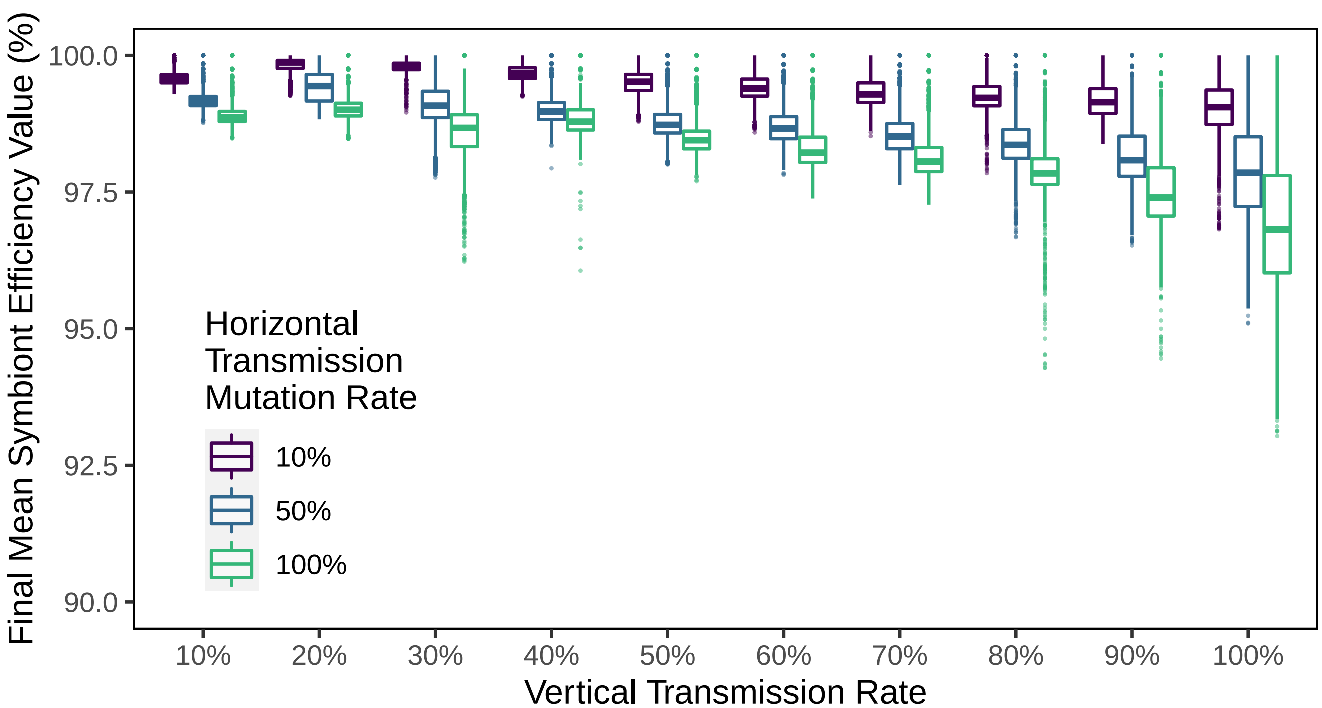

Figure 4: **Mean efficiency value of symbionts at final timestep.** Efficiency value is the percentage of resources a symbiont is able to convert to energy for use in reproduction. In all treatments, average efficiency value did not decrease below 90%.

Results and Discussion

To determine the effect of a higher mutation rate during horizontal transmission (HTMR) on the evolution of mutualism, we enabled hosts and endosymbionts to evolve when the mutation rates during horizontal and vertical transmission were both 10% and when the mutation rate during horizontal transmission was 50%. We tested the effect at vertical transmission rates from 10-100% at 10% intervals. We then also explored whether the effect of a higher mutation rate during horizontal transmission would increase when the mutation rate during horizontal transmission was raised further to 100%. All treatments started with populations of hosts and symbionts with random starting interaction values and symbiont's had starting efficiency of 100% and evolution proceeded for 10,000 timesteps. Hosts were restricted to having at most one endosymbionts, keeping the multiplicity of infection to at most 1.

Increased HTMR Selects for Increased Mutualism at Intermediate Vertical Transmission Rates

We first determined the effect of an increased horizontal transmission mutation rate by comparing the degree of mutualism that symbionts evolve when HTMR is 10 and 50% and all other mutation rates are held at 10% across vertical transmission rates.

As shown in Figure 3, when the vertical transmission rate is low (10-20%) or high (70-100%), a higher HTMR does not have a meaningful impact on the final degree of mutualism that evolves in the symbionts (some treatments have a significant difference, however the effect size is not meaningful). The result is probably due to the fact that the dominant selection pressure at these extreme vertical transmission rates is from either rarely or usually vertically transmitting. Specifically, in agreement with previous work (Vostinar and Ofria, 2019), when vertical transmission rate is high, symbionts evolve to donate nearly all of their resources to their hosts, losing the ability to horizontally transmit and thus negating any effect of a higher mutation rate during horizontal transmission. Conversely, when vertical transmission rate is quite low, even though vertical transmission may be beneficial, it is such a rare occurrence that symbionts are selected to be extremely parasitic anyway.

However, at intermediate vertical transmission rates of 30%, 40%, 50%, and 60%, an HTMR of 50% results in significantly more mutualistic symbionts than when the HTMR is the same as the other mutation rates at 10% ($p < .005$ for all comparisons).

As shown in Figure 4, for all treatments, the mean efficiency values of the symbionts remains above 95%, indicating that these results are not due to mutational breakdown. In agreement with the theory around a lack of purifying selection on endosymbionts at very high vertical transmission rates (O'Fallon, 2008), the lowest mean efficiency values are actually found at the highest vertical transmission rates. These results demonstrate that the Dirty Transmission Hypothesis does not conflict with the predictions of endosymbiont 'de-evolution' due to a lack of purifying selection at high vertical transmission rates.

These results indicate that when the chance of vertical transmission is near 50%, and therefore the selection pressure from vertical transmission or lack thereof is weaker, a higher HTMR can tip the balance towards mutualism, supporting the Dirty Transmission Hypothesis. Specifically, when the vertical transmission rate is 30%, a higher HTMR makes mutualism possible where it otherwise wouldn't be and when the vertical transmission rate is 50 or 60%, a higher HTMR pushes mutualism from a possibility to a near certainty.

Impact of 100% HTMR on Evolution of Mutualism

To explore the full effects of a higher mutation rate during horizontal transmission, we also determined the effect of a 100% HTMR. We focused on the vertical transmission rates below 60% due to the lack of meaningful impact above that rate due to the low amount of horizontal transmission. We again started host and symbiont populations at random interaction values, enabled evolution for 10,000 timesteps, and measured the average interaction value of the symbionts after evolution.

As shown in Figure 3, an HTMR of 100% does not lead to a significant difference in the amount of mutualism evolved compared to an HTMR of 50% ($p >= 0.05$). Note that when the vertical transmission rate is 40%, the individual treatment difference appears significant, however it does not remain significant when corrected for multiple comparisons, as discussed in the Methods. This result indicates that the effect of an increased mutation rate during horizontal transmission does not necessarily depend on the amount of mutation rate increase.

Differential Effects of Increased HTMR on Host-Associated Traits

An increased rate of mutation during horizontal transmission impacts both the symbiont's interaction value (i.e. its host-associated traits) and its efficiency value (i.e. its adaptive traits that do not impact its interaction with the host). To determine the impact of increased HTMR on each of these traits individually, we repeated the previously described experiments with the mutation rate of the efficiency value held constant at 10%. Therefore, only the symbiont's interaction value was subject to the increased mutation rate during horizontal transmission.

As expected, and shown in Figure 5, when the efficiency value is not under increased HTMR, the final evolved efficiency values remain above 95% in all treatments. However, as shown in Figure 7, the final interaction value of symbionts is still impacted by the increased HTMR at intermediate vertical transmission rates. When the increased HTMR

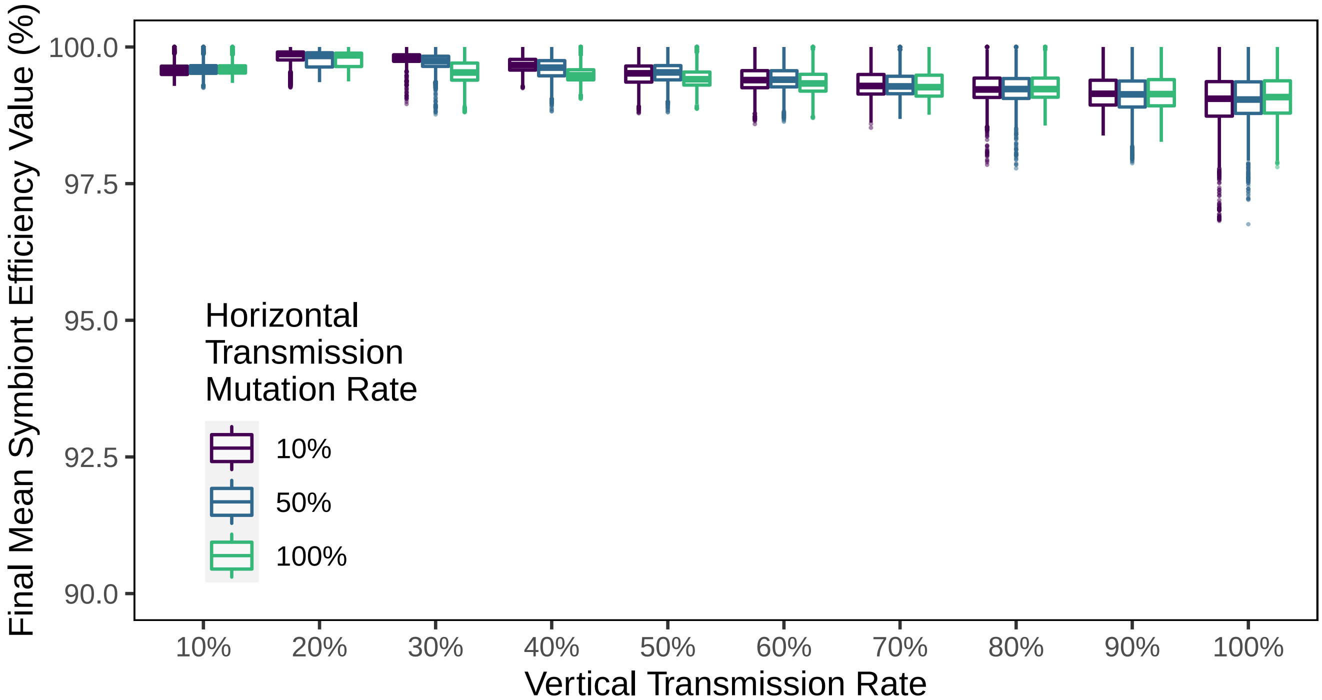

Figure 5: **Mean efficiency value of symbionts at the final timestep when only the interaction value was under an increased mutation rate during horizontal transmission and the HTMR of efficiency value was held at 10%.** In all treatments, average efficiency value did not decrease below 95%.

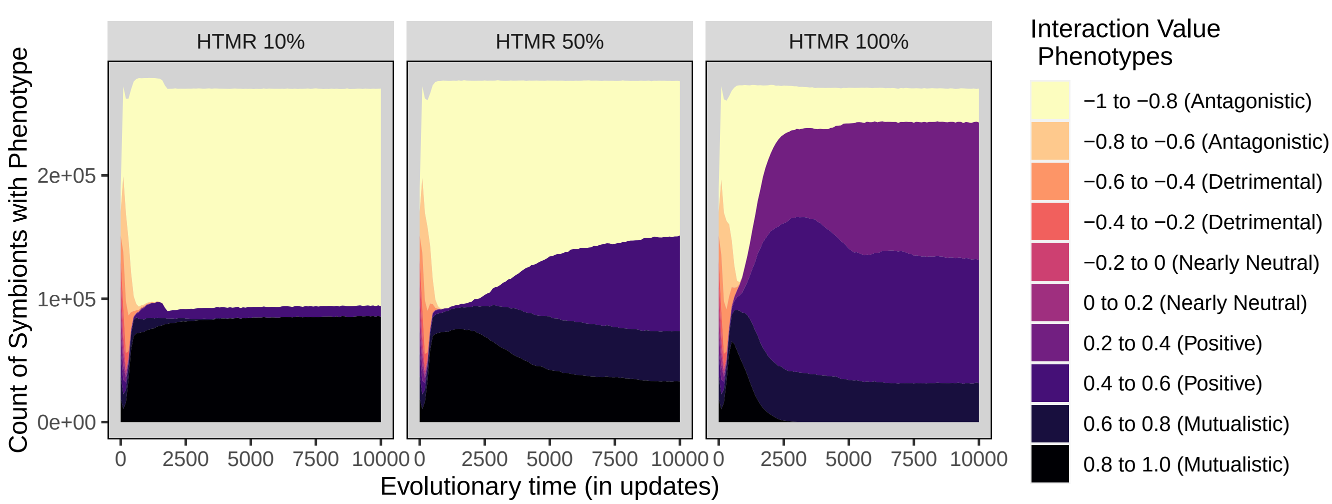

Figure 6: **Count of symbiont phenotypes over time at a vertical transmission rate of 30% and when only the interaction value is subject to increased mutation during horizontal transmission.** All other mutation rates were held constant at 10%.

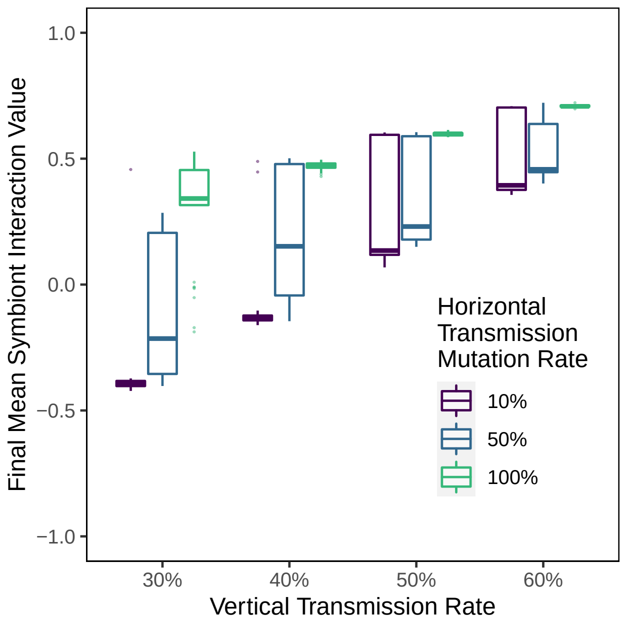

Figure 7: **Mean symbiont interaction value at intermediate vertical transmission rates when the rate of mutation during horizontal transmission was increased only for the interaction value.** The mutation rate for symbiont efficiency value, host traits, and the mutation rate during vertical transmission was held at 10%.

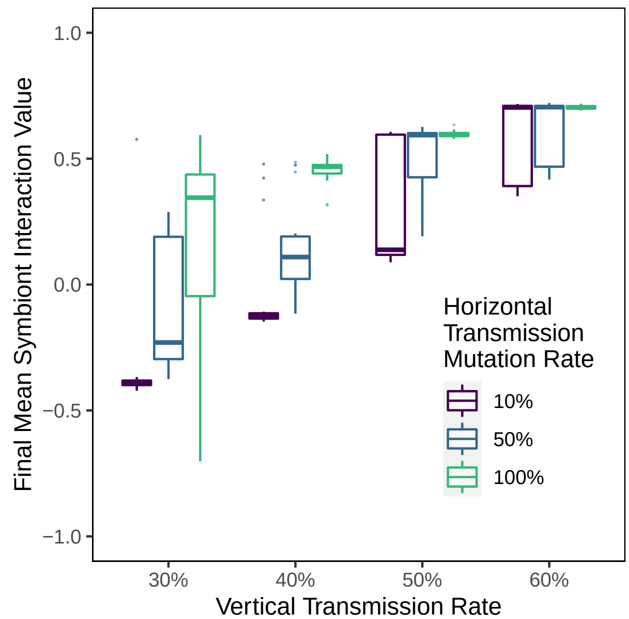

Figure 8: **Mean symbiont interaction value across vertical transmission rates when the rate of mutation during horizontal transmission was increased only for the efficiency value.** The mutation rate for symbiont interaction value, as well as during vertical transmission and host reproduction, was held at 10%.

only effects the symbiont's host-associated trait (interaction value), 100% HTMR selects for a significantly higher final median symbiont interaction value at vertical transmission rates of 30, 40, 50, and 60% (all $p < 0.05$). As an example, Figure 6 shows the distribution of symbiont phenotypes over time at each HTMR when vertical transmission is 30%, demonstrating that the populations are stably dominated by mutualistic symbionts when HTMR is 100%, but not at the lower HTMR values. Note that the final median interaction values are not significantly different between the following treatments when the HTMR is 30% and the HTMR is 50%: 1) when both traits are subjected to increased HTMR and 2) only the interaction value is ($p > 1$), meaning that the difference seen in this treatment is not due to a change in the degree of mutualism at 50% HTMR. These results indicate that when only host-associated traits are impacted by increased HTMR, a further increase from 50% to 100% does have an impact at intermediate vertical transmission rates. They also demonstrate that the effect on the host-associated trait of the interaction value contributes to the overall increased rate of mutualism, but does not fully explain it.

Effects of Increased HTMR on Non-Host Associated Traits

Finally, we investigated the effect of the increased mutation rate during horizontal transmission on the symbiont's non-host associated trait: the efficiency value. We held the HTMR of the symbionts' interaction value constant at 10% and conducted the same experiments with HTMR levels of 10, 50, and 100% on the efficiency value.

As shown in Figure 8, the effects of increased HTMR on the efficiency value are generally qualitatively consistent with the effects of overall increased HTMR when vertical transmission rates are 60% or above, or 20% or below. However, at 30 and 40% vertical transmission rates, 100% HTMR leads to significantly more mutualistic symbionts than at 50% HTMR (all $p < 0.05$). Specifically, when vertical transmission rate is 30%, an HTMR of 100% leads to a median interaction value of 0.34, whereas when HTMR is 50%, the median interaction value is -0.21. These results, when combined with the previous section, indicate that the increased mutualism evolved during higher HTMR at intermediate vertical transmission rates is due to both the effect on the interaction value and the efficiency value. However, when both traits are under an increased mutation rate at 50% HTMR, the combined effect is qualitatively equivalent to a HTMR of 100% on only one of the traits. This means that if only a host-associated or non-host-associated trait is under increased mutational load, there can be increased selection for mutualism at the most extreme HTMR.

Conclusion

In this work, we presented a novel mechanism for the evolution of mutualism, termed the Dirty Transmission Hypothesis. Specifically, we demonstrated that high mutation rates associated with horizontal transmission can select for higher levels of mutualism when vertical transmission rates are at intermediate values. We also examined the effect of extreme mutation rates during horizontal transmission and the contributing effects of increased mutation rates on host-associated and non-host-associated symbiont traits. We demonstrated that the increased mutualism that is evolved when the rate of mutation during horizontal transmission increases is due to the combined effects on symbiont traits that are associated with the host and symbiont traits that are independent of its interaction with the host.

There are many future directions to explore regarding the effect of the Dirty Transmission Hypothesis. This work focused on single-infecting obligate endosymbionts, however multi-infection and symbionts that are capable of surviving outside of the host are common occurrences in natural systems and therefore fertile ground for further exploration.

Many natural systems have vertical transmission rates that appear insufficient to select for mutualistic behavior and yet mutualism is found in those systems. There are many mechanisms that can lead to increased selection for mutualism, however they often require organisms capable of complex behavior. Here, we have experimentally demonstrated that the simple environmental effect of higher mutation rate during horizontal transmission can directly select for increased mutualism at realistic vertical transmission rates. This work contributes to our understanding of under what conditions mutualism can be expected to evolve and persist, and indicates how we may be able to predict and control its evolutionary trajectory in symbiotic systems vital to human health and society.

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