Endosymbiosis or Bust: Influence of Ectosymbiosis on Evolution of Obligate Endosymbiosis

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Abstract

Endosymbiosis, symbiosis in which one symbiont lives inside another, is woven throughout the history of life and the story of its evolution. From the mitochondrion residing in almost every eukaryotic cell to the gut microbiome found in every human, endosymbiosis is a cornerstone of the biological processes that sustain life on Earth. While endosymbiosis is ubiquitous, many questions about its origins remain shrouded in mystery; one question in particular regards the general conditions and possible trajectories for its evolution. Modern science has hypothesized two possible pathways for the evolution of mutualistic endosymbiosis: one where an obligate antagonism is co-opted into an obligate mutualism (Co-Opted Antagonism Hypothesis), and one where a facultative mutualism evolves into an obligate mutualism (Black Queen Hypothesis). We investigated the viability of these pathways under different environmental conditions by expanding on the evolutionary agent-based system Symbulation. Specifically, we considered the impact of ectosymbiosis on *de novo* evolution of obligate mutualistic endosymbiosis. We found that introducing a facultative ectosymbiotic state allows endosymbiosis to evolve in a more diverse set of environmental conditions, while also decreasing the evolution of endosymbiosis in conditions where it can evolve independently.

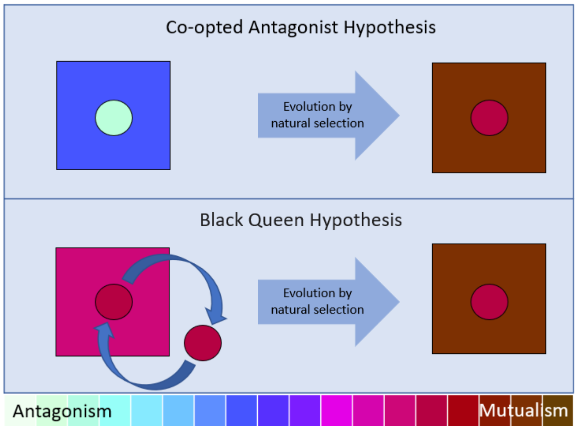

Figure 1: **Two of the posited hypotheses for the evolution of endosymbiosis.** Squares are host organisms and circles are symbionts. Color indicates whether the organism is antagonistic (pale green to blue) or mutualistic (purple to brown) towards its partner.

Introduction

Endosymbiosis has played a crucial role in the evolutionary history of eukaryotes, as well as the evolution of life as a whole (Martin et al., 2015).[1] In particular, the evolution of endosymbiosis drove the major evolutionary transitions involving plastids (de Vries and Archibald, 2017) and mitochondria. The endosymbiotic acquisition of mitochondria provided so much chemical energy that it encouraged a wide expansion of the eukaryotic clade (Archibald, 2015; Zachar and Boza, 2020). Furthermore, humans are hosts to many endosymbionts; hence, analyzing their evolution and interaction with hosts is necessary to understanding the human system (Eloe-Fadrosh and Rasko, 2013; Perotti et al., 2007). Whether antagonistic or mutualistic, endosymbiotic relationships impact the population diversity and the complexity achieved by host species (Vostinar et al., 2021) as involved members undergo coevolution (Lazcano and Peretó, 2017).

While the precise origins of every obligate mutualistic endosymbiosis necessarily remain unclear, two of the major hypothesized pathways are: the Co-Opted Antagonist Hypothesis (Johnson et al., 2021) and the Black Queen Hypothesis (Morris et al., 2012) (Figure 1). The Co-Opted Antagonist Hypothesis proposes that an obligate mutualism evolves when an antagonistic relationship is co-opted into a relationship that benefits both the host and endosymbiont, whereas the Black Queen Hypothesis suggests that a pre-existing facultative mutualism evolves into an obligate mutualism because one or both partners lose functionality required to remain independent. The relationship between these hypotheses is not an obligate dichotomy; it is likely that they have both contributed to the evolution of various endosymbioses. However, it is unknown which path is more likely and how

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[1] Symbiosis is a close and sustained relationship between individuals of different species (Lewin, 1982). Endosymbiosis is a specific form of symbiosis in which one organism lives inside the body or cells of the other.

they may interact in a co-evolving population.

The timescales and resources required to observe co-evolutionary dynamics in a traditional laboratory environment inherently hinder their investigation. Even the fastest evolving microbial systems still require weeks, months or years to achieve the necessary evolutionary timescales. Further, current technology lacks the ability to perfectly control every potential confounding variable and perform data collection at the level of each individual organism. However, interactions between individual organisms in complex and varied populations are necessary to investigate symbiotic dynamics, making traditional population-level analytical modeling also insufficient. Therefore, we utilized and expanded upon Symbulation – an evolutionary agent-based platform designed to explore symbiotic relationships – to investigate the trajectories of co-evolving populations during the *de novo* evolution of endosymbiosis.

Specifically, the question that this investigation is centered around is: what are the conditions under which a mutualistic obligate endosymbiotic relationship can evolve and how do facultative and antagonistic intermediate stages impact that evolution? We determined that ectosymbiosis 1) expands the conditions in which endosymbiosis can evolve and 2) decreases the evolution of endosymbiosis in conditions where it would independently evolve.

Methods

To investigate the *de novo* evolution of obligate endosymbiosis, we used Symbulation, an open-source agent-based modeling platform for the study of symbiosis (Vostinar, 2021) that is built upon Empirical (Ofria et al., 2020). As shown in Figure 2, we created a virtual world with the following:

1. the necessary elements for evolution via natural selection (time, variation, competition, inheritance) for a population of 'hosts' and a population of 'symbionts' (whether they engaged in symbiosis or not),
2. the possibility of an interaction between an individual host and symbiont that was anywhere along a spectrum between parasitism/antagonism and mutualism,
3. the possibility for a free-living symbiont to infect a host and become an endosymbiont, and
4. a limit of at most one symbiont able to interact with each host.

Specifically, each experiment began with a full population of 10,000 hosts and a population of approximately 7,000 free-living symbionts. As shown in Figure 2, hosts and free-living symbionts exist in distinct but parallel populations with corresponding locations. This representation is necessary because limited space (only 10,000 locations are in the world) is the main source of competition (resources are set to unlimited), however for the questions of interest in this work, hosts and free-living symbionts should not compete directly with each other (in the same way that humans are rarely directly competing with bacteria for limited resources). This world structure allows for the hosts and symbionts to not compete with each other for space in the world. Instead, hosts compete only with other hosts, and symbionts with other symbionts. Additionally, hosts and symbionts can exist completely independently of each other, enabling us to also explore the possibility that, in certain environmental conditions, endosymbiosis will not evolve at all.

During these experiments, each organism receives a set amount of resources per timestep from the world, which varies by treatment. Upon accruing sufficient resources, both hosts and symbionts can reproduce with a chance of mutation. Hosts reproduce once they have collected 600 resources, free-living symbionts when they have collected 300 resources, and the resource quantity required for endosymbiont reproduction varies depending on the *transmission mode*, as discussed in the following paragraph. All reproduction is asexual and an offspring inherits its genome from its parent with mutations. A mutation of some kind will occur 100% of the time, but the size of the mutation varies. The mutation size is selected from a Gaussian distribution with a mean of 0 and standard deviation of 0.05.[2] Reproduction by hosts and free-living symbionts sends the offspring to a random world position. If an organism was occupying that space in the world, the offspring kills the former inhabitant (and its endosymbiont, if it has one). Both hosts and symbionts can also die of old age, 60 timesteps for hosts and 30 timesteps for symbionts, regardless of whether they are endosymbionts or free-living.

Endosymbionts have two possible transmission modes. First, when a host reproduces, the endosymbiont might *vertically transmit* an offspring based upon the user-configured *vertical transmission rate*, which is 50% by default. When a host reproduces, a random number between 0 and 1 is checked against the user-configured vertical transmission rate. In our experiments, we varied vertical transmission rate from 0 to 100% at 10% intervals. If that number is less than the vertical transmission rate and the endosymbiont has sufficient resources (200), the endosymbiont will also reproduce and transmit its offspring directly into the host offspring before the host offspring is dispersed as normal. The other possibility is *horizontal transmission*, which occurs when an endosymbiont acquires enough resources to reproduce without the help of its host (300). The endosymbiont's offspring exits the host and becomes a free-living symbiont in a random location of the world; the offspring can later attempt to become an endosymbiont by infecting a host. Note that a high vertical transmission rate does not mean that endosymbionts will exclusively transmit vertically – in situations where the endosymbionts reach 300

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[2] If the mutation causes the trait to go outside of the fixed bounds, the trait is set to the nearest bound.

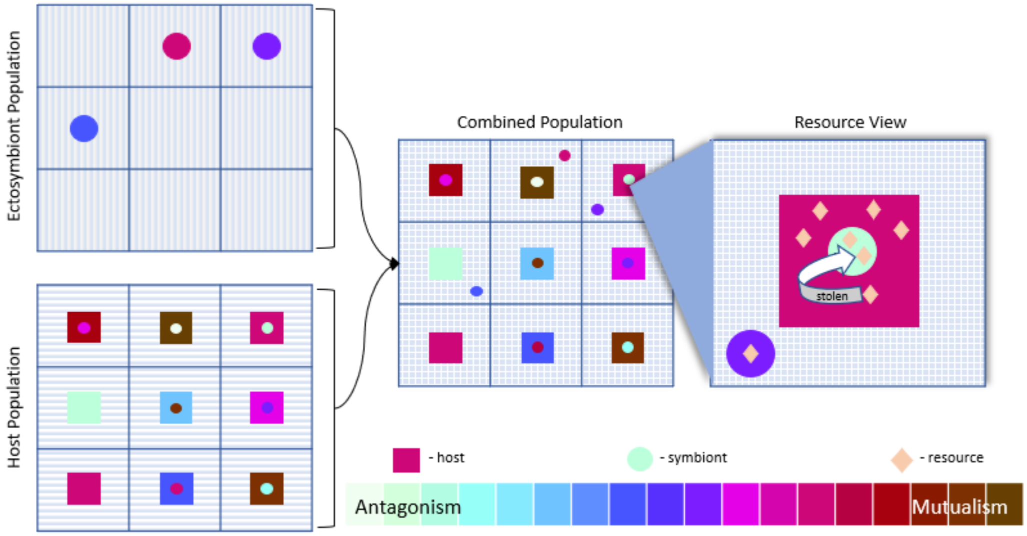

Figure 2: **A general overview of the Symbulation system.** Opaque squares are host organisms, circles are symbionts, and diamonds are resources. Color of hosts and symbionts indicate phenotype, ranging from antagonistic (pale green to blue) to mutualistic (purple to brown) towards a potential partner. The system is implemented with parallel populations of hosts and free-living symbionts, such that hosts and symbionts do not compete for limited space between species. However, free-living symbionts are able to infect hosts in the corresponding location of the parallel population.

resources more quickly than the hosts reach 600 resources, such as when highly parasitic symbionts infect weakly antagonistic hosts (and steal most of the host's incoming resources), they will still horizontally transmit. In mutualistic relationships, however, if vertical transmission rate is high then the principle transmission mode will almost certainly be vertical, as hosts will accrue resources more quickly than their endosymbionts.

Each organism has a single floating-point number that represents its behavior on the antagonism to mutualism spectrum, which we will refer to as the 'interaction value.'[3] All hosts and symbionts begin every experiment with interaction values of 0, which assumes that they have not previously co-evolved together. Interaction values can span from -1 – representing antagonism (parasitism/defensiveness) – to 1 – representing mutualism. The further their interaction value is from 0, the more extreme the behaviour they exhibit.

An antagonistic host spends a portion of its incoming resources (based on its interaction value) on defense, while an antagonistic symbiont attempts to steal resources from its host. Symbionts that are less antagonistic than their hosts (*i.e.* if the host interaction value is more negative) fail to steal any resources and their hosts retain whatever proportion of resources they didn't spend on defense. If, however, a symbiont is more antagonistic than a host, it successfully overpowers the host's defenses and steals a proportion of the resources that weren't already spent on defense. The proportion stolen is based on the difference between the symbiont's interaction value and the host's. For example, if a symbiont has an interaction value of -1 and a host has an interaction value of -0.1, and the host receives 100 resources per update, then the host would spend 10 resources on defense (and those resources would be unavailable for either the symbiont or host to use), leaving 90 resources. The symbiont would then steal 81 of the remaining resources, and the host would keep the final 9 resources for its own reproduction.

When mutualistic, a host donates a portion of its resources to its symbiont based on the host's interaction value, while a mutualistic symbiont sends a portion of its resources back to its host based on the symbiont's own interaction value; the resources returned to the host by the symbiont are multiplied by a user-configured synergy factor of 5. For example, a mutualistic host with an interaction value of 0.5 might have a mutualistic symbiont with an interaction value of 0.5 as well. The host would receive 100 resources and donate 50 of them to the symbiont. The symbiont would then keep half (25) of the resources and donate back the other half, with the donated portion multiplied by the synergy (5). Therefore, at the end of the resource distribution process, the host would have 175 resources, and the symbiont would have 25 resources.

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[3] Previous work using Symbulation used the term 'resource behavior value' instead.

By default, symbionts can only interact with hosts through endosymbiosis, i.e. they must have infected the host already to interact with it. However, in parts of this work we also allow for *ectosymbiosis*. Ectosymbiosis will occur between a host and a symbiont in corresponding locations of their respective populations, but only if the host does not have an endosymbiont (this restriction is to remove the confounding factor of a host being able to have two symbionts when ectosymbiosis is enabled, but is configurable and could be relaxed in future work). In an ectosymbiotic relationship, resource distribution (mutualistic and parasitic/defensive behavior) unfolds identically to endosymbiosis.

If hosts or free-living symbionts have no partner, they will still spend resources attempting their symbiotic behavior (attempting to steal, investing in defense, or donating resources out) but incur no benefit. Thus, unless symbiosis is beneficial, an interaction value of 0 is optimal. This penalty decreases random drift of the interaction value in the absence of the partner species and means that interaction values that deviate from 0 are likely meaningful. Note that ectosymbionts can be considered facultative because they are able to survive outside of and without a host, though they may still suffer a fitness penalty if they have evolved to rely on a host.

We implemented an additional symbiont trait, *infection chance*, governed by another floating point number that can be between 0 (never try to infect) and 1 (always try to infect). At the beginning of an experiment, infection chance is 0 for all symbionts, but is under the same inheritance and mutation regime as the interaction value.

At each timestep, each free-living symbiont has a chance to attempt to infect a host based on its infection chance. If it decides to attempt infection, it attempts to enter the host with the matching location to its own in the host population. The infection can still fail if there isn't a host at that location, the host already has an endosymbiont (because only one endosymbiont is allowed per host in these experiments), or based on the user-configured infection failure rate. If infection is unsuccessful the aspiring endosymbiont is killed and removed from the symbiont population.

All experiments were run for 100,000 timesteps with 31 replicates per treatment. We used R (R Core Team, 2020) and the ggplot2 (Wickham, 2016) and viridis (Garnier et al., 2021) packages for all plots. For all statistical analysis we used Wilcoxon rank-sum tests and Bonferroni corrections for multiple comparisons. All code to recreate the experiments and analysis, along with data and supplementary materials, are available at `https://github.com/anyaevostinar/Evolution-of-Endosymbiosis-Paper`.

Results and Discussion

To investigate the *de novo* evolution of symbiosis we conducted three sets of experiments: 1) determining the degree to which ectosymbiosis evolves when endosymbiosis is prohibited, 2) investigating the evolution of endosymbiosis directly from a free-living ancestor, and 3) determining how the possibility of ectosymbiosis impacts the evolution of endosymbiosis. In each set of experiments, we started with a population of hosts and free-living 'symbionts'. Note that we refer to the two species as 'host' and 'symbiont' even when they are not engaged in a symbiosis for the sake of clarity. Due to the possible effect of resource availability, in all experiments, we varied the amount of resources received by hosts and free-living symbionts at each timestep. The resource amounts were 10, 50, 100, 500, or 1000 resources per organism per update. We tested each pairwise combination of resource amounts for each species.

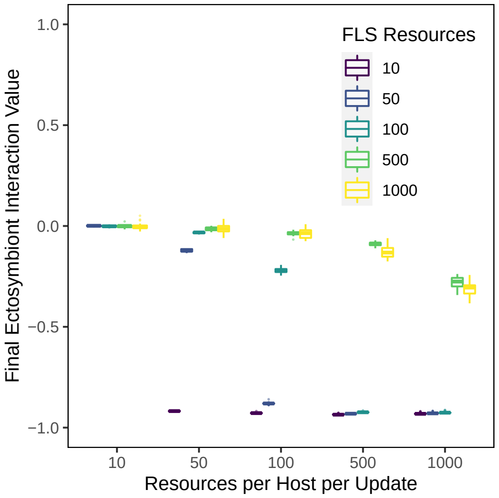

Figure 3: **Free living ectosymbiont interaction value at final timestep when endosymbiosis was prohibited.** FLS Resources are the resources distributed to free-living symbionts each timestep. When all organisms received 10 resources/timestep, the free symbiont population went extinct.

Evolution of Ectosymbiosis in the Absence of Endosymbiosis

We first investigated whether our system would evolve significant ectosymbiosis in the absence of the possibility of endosymbiosis. We ran simulations were the endosymbiont limit was set to 0, therefore, ensuring that no endosymbiosis was possible. We determined the amount of ectosymbiosis based on the interaction values of the hosts and symbionts. If the organisms evolved to rely on ectosymbiosis, their interaction values would deviate from 0.

As shown in Figure 3, the amount of ectosymbiosis that evolved depended on the resource amounts received by the hosts and symbionts. When hosts received only 10 resources per update, no meaningful amount of symbiosis evolved, and the symbionts went extinct when they also received only 10 resources per update. Increasing the amount of resources

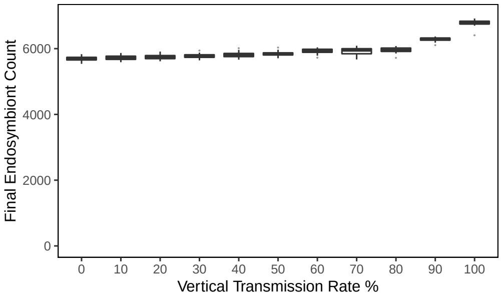

Figure 4: **Endosymbiont counts at final timestep when ectosymbiosis was prohibited across vertical transmission rates.** Hosts received 100 resources per organism per update and free-living symbionts received 50 resources per organism per update.

given to hosts generally led to increased levels of parasitism among the symbionts. For example, when symbionts received 100 resources per update, they evolved to be significantly more parasitic when hosts received 100 resources per update than when the hosts received only 10 resources per update ($p < 0.005$).

These results indicate that the evolution of ectosymbiosis in this system depends on the amount of resources available to both hosts and symbionts. They also indicate that, in the absence of vertical transmission through endosymbiosis, mutualistic symbiosis does not evolve in this system.

Endosymbiosis Can Evolve Directly From Free-Living Ancestor

We next determined which environmental factors favor the evolution of *de novo* endosymbiosis, by running simulations with 50% vertical transmission and varying the resources received by free-living symbionts and hosts.

We conducted two control treatments where endosymbiosis was not beneficial. In the first control we held the host's interaction value at -1, meaning that hosts invested all of their resources into defense (and therefore we also prevented them from dying of old age because they were unable to reproduce). In this control, symbiont interaction values remained at 0 as expected (data in supplemental materials). In the second control, we set the infection failure chance at 100%, meaning symbionts could never successfully infect a host and engage in symbiosis. As expected, we again found that interaction values and infection chance remained at 0 (data in supplemental materials).

We next examined the impact of vertical transmission rate on the evolution of endosymbiosis. The evolved interaction value of endosymbionts agreed with previous work (Vostinar and Ofria, 2019). However, as shown in Figure 4, vertical transmission rate did not have a meaningful effect on the final number of endosymbionts.

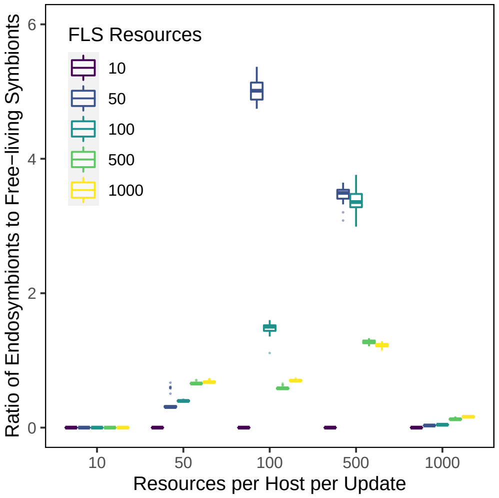

Figure 5: **Ratios of endosymbionts to free-living symbionts at final timestep when ectosymbiosis was prohibited.** When free-living symbionts received 10 resources per update, all symbiont populations went extinct.

We then determined how the amount of resources received by hosts and free-living symbionts impacts the evolution of endosymbiosis when ectosymbiosis is not possible. As shown in Figure 5, the amount of resources accrued by hosts and free-living symbionts impacts the relative amount of endosymbionts compared to free-living symbionts. In general, intermediate amounts of resources for both hosts and free-living symbionts lead to the highest ratio of endosymbionts to free-living symbionts. Specifically, when free-living symbionts receive 50 resources per symbiont per update and hosts receive 100 resources per organism per update, the ratio of endosymbionts to free-living symbionts is 5.02, while the treatment with the next highest ratio is significantly lower at 3.47 endosymbionts/free-living symbionts when hosts receive 500 resources per update and symbionts still receive 50 resources/update/organism ($p < 0.005$)

The amount of resources received by hosts and free-living symbionts also impacted the final behavior of the endosymbionts, as shown in Figure 6. At resource amounts of 10, 50 or 100 for endosymbionts and 50 or 100 for hosts, endosymbionts generally remained neutral to the host, whereas at resource/update amounts of 50 for free-living symbionts and 500 for hosts, endosymbionts evolved to mostly be parasitic, with an average interaction value of -0.767. When the hosts received 500 resources/update and free-living symbionts received 500 or 1000 resources/update, endosymbionts evolved to be significantly more mutualistic, with average interaction values of 0.293 and 0.325, respectively ($p < 0.00005$ for both).

These results show that host resource levels significantly impact the evolution of *de novo* endosymbiosis, and that there is an ideal intermediary resource level that favors the

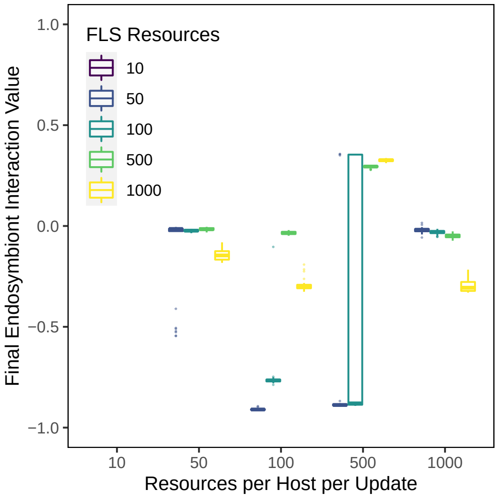

Figure 6: **Endosymbiont interaction value at final timestep when ectosymbiosis was prohibited.** When hosts were given 10 resources per update, the symbiont population died out.

strongest levels of endosymbiosis. When resource levels are too low (10), symbionts go extinct, and so endosymbiosis does not evolve. Conversely, when resource levels are too high (1000), hosts are able to undergo rapid proliferation, making endosymbiosis unfavorable; thus it does not evolve. In addition, the amount of resources received by both species influences the nature of the symbiotic relationship, with a limited range of values selecting for mutualism. The mechanisms underlying this range are worthy of future study.

Does the option of ectosymbiosis increase the evolution of endosymbiosis?

To investigate if the possibility of ectosymbiosis increases the evolution of endosymbiosis, we ran simulations with both ectosymbiosis and endosymbiosis permitted with the same range of resources received by both species.

As shown in Figure 7, the effect of ectosymbiosis on the evolution of endosymbiosis varies dramatically across environmental conditions. For example, when free-living symbionts receive 50 resources/update and hosts receive 100 resources/update, ectosymbiosis significantly *decreases* the rate of endosymbiosis ($p < 0.005$). Conversely, when hosts receive 1000 resources per host per update, permitting ectosymbiosis significantly increases endosymbiosis across all free-living symbiont resource levels (all p-values < 0.0005)

These results indicate that ectosymbiosis enables the survival of symbionts in 1) conditions that are not ideal for symbionts, such as when free-living symbionts receive a low amount of resources, and 2) conditions that are not ideal for endosymbiosis, such as when hosts are able to reproduce rapidly. When the symbionts can survive in these conditions, they can then evolve towards endosymbiosis. However, counter to our hypothesis, in more ideal conditions, when endosymbiosis is able to evolve without ectosymbiosis, the option of ectosymbiosis decreases the degree to which endosymbiosis evolves. These results therefore suggest that endosymbioses will evolve in a more diverse set of conditions when ectosymbiosis is possible, however endosymbiosis may evolve to a lesser degree in ideal conditions.

What path do endosymbionts take to mutualism?

Finally, we investigated which of the two hypothesized pathways mutualistic endosymbionts took during evolution. To answer this question, we measured the complete phylogeny of the population, defining taxonomic units based on a discretization of the space of possible interaction values into 4 distinct bins. For a more thorough discussion of our phylogeny tracking methodology, see (Dolson et al., 2020). We then extracted the full lineage of the dominant (*i.e.* most numerous) taxonomic unit at the end of each replicate run. Finally, we compared the dominant lineages under our three experimental conditions: (1) endosymbiosis only, (2) ectosymbiosis only, and (3) endosymbiosis and ectosymbiosis both possible. Note that the Black Queen Hypothesis pathway was only possible when ectosymbiosis was enabled.

As shown in Figure 8, when only ectosymbiosis was permitted, all dominant symbiont lineages were intermittently parasitic. Conversely, when only endosymbiosis was permitted, most dominant symbiont lineages ended in a mutualistic phenotype, but spent some evolutionary time somewhat parasitic. However, the degree to which the dominant symbiont lineage was parasitic depended on the amount of resources each free-living symbiont received at each timestep. Specifically, when free-living symbionts received 500 resources/organism/update, no symbiont lineage spent any time in the extremely parasitic phenotype, but when the free-living symbionts received 1000 resources/organism/update, most (26/31 replicates) symbiont lineages were extremely parasitic for a period of their evolutionary history.

Finally, when ectosymbiosis and endosymbiosis were both possible, all symbiont lineages spent time in the somewhat parasitic phenotype space during their evolution. However, the degree of parasitism again depended on the amount of resources received by the free-living symbionts. When free-living symbionts received 500 resources/organism/update, 1/31 lineages spent evolutionary time in the extremely parasitic phenotype state. Conversely, when free-living symbionts received 1000 resources/update/organism, 19/31 symbiont lineages spent time in the extremely parasitic state.

These results suggest that the co-opted antagonist hypothesis is the dominant evolutionary pathway towards mutualism under these conditions. All final dominant symbiont lineages were historically parasitic to some degree. However, the degree of parasitism and the amount of evolutionary time spent parasitic was strongly influenced by the amount of re-

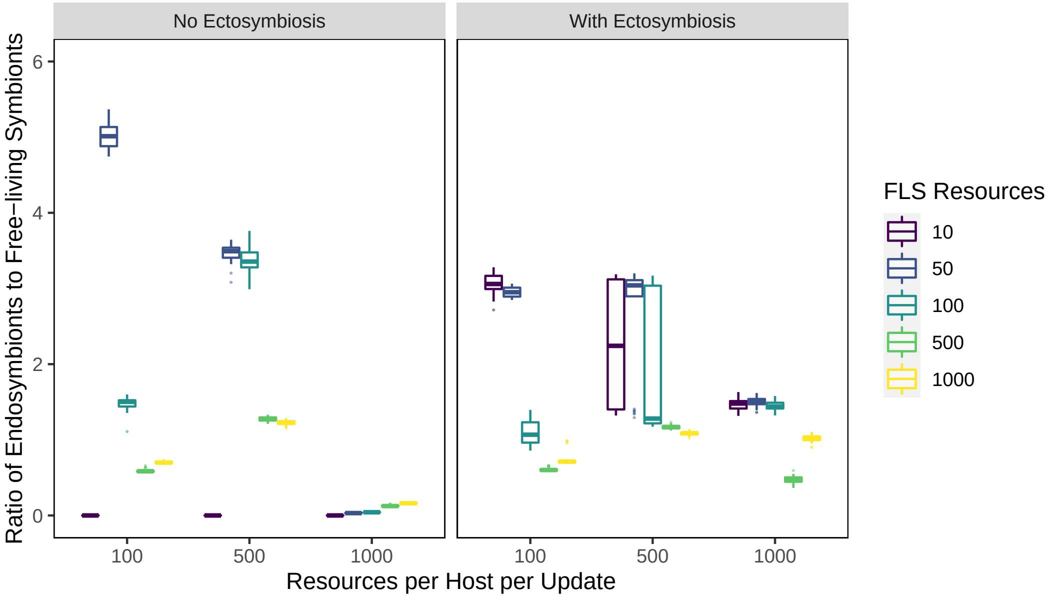

Figure 7: **Ratios of endosymbionts to free-living symbionts at final timestep with and without ectosymbiosis.** Resources distributed to hosts and free-living symbionts each timestep were varied at 10, 50, 100, 500, and 1000 for each species (host resource/update amounts of 10 and 50 were not meaningfully different and so are not shown here but are included in the supplemental material).

sources the free-living symbionts received and whether ectosymbiosis was possible. Specifically, when symbionts receive more resources from the world, they are more likely to engage in stronger parasitism, and for a longer period of evolutionary time. Further, the possibility of an ectosymbiotic intermediary stage reduces the duration of evolutionary time that lineages spend in a strongly parasitic state, and hastens the evolution of mutualism. Presumably, the presence of ectosymbiosis means that a population can undergo lower-commitment mutations regarding symbiotic behavior while still receiving resources from the world. Thus such populations are less reliant upon coevolution from their symbiotic partners, in support of the Black Queen Hypothesis. However, while the presence of endosymbiosis almost always selects for mutualism, when only ectosymbiosis is possible the dominant lineages are likely to be somewhat parasitic, though selection is weaker overall.

Conclusion

We have shown that introducing the possibility of an ectosymbiotic intermediary stage into the evolution of endosymbiosis 1) diversifies the environmental conditions in which endosymbiosis is able to evolve, and 2) lessens the evolution of endosymbiosis in conditions where it can evolve independently. Further, within the conditions where we conducted extended phylogenetic analysis, most mutualistic symbionts descended from parasitic ancestors. However, adding the capacity for ectosymbiosis in addition to endosymbiosis promotes faster evolution of mutualism, but ectosymbiosis alone does not enable mutualism to evolve. Therefore, this work supports the Co-Opted Antagonist Hypothesis as the dominant evolutionary trajectory in our system, with Black Queen dynamics present to a lesser extent.

There are many other factors that influence the evolution of endosymbiosis and should be explored in future work. Specifically, many symbiotic systems allow for multiple symbionts (endo- and ecto-) to infect and interact with the same host. Symbulation would be an ideal system to further expand for the investigation of the effect of multi-infection on the *de novo* evolution of endosymbiosis. Further, many systems have elements of host and symbiont partner choice, which likely also would impact the evolutionary trajectory of both partners. Finally, Symbulation will be a valuable system for developing and testing metrics for analyzing the phylogenetic structure of mutualistic endosymbionts.

Endosymbiosis is a biological phenomenon that has been essential for creating and sustaining eukaryotic life. Therefore, by uncovering the conditions necessary for *in silico* endosymbiosis to evolve, we have unveiled possible evolutionary pathways that *in vivo* endosymbionts may have taken. This work contributes to the story of why Earth's multicellular terrestrial life has persisted fruitfully, predictions of extraterrestrial life, and can be used to predict future evolution of vital endosymbionts.

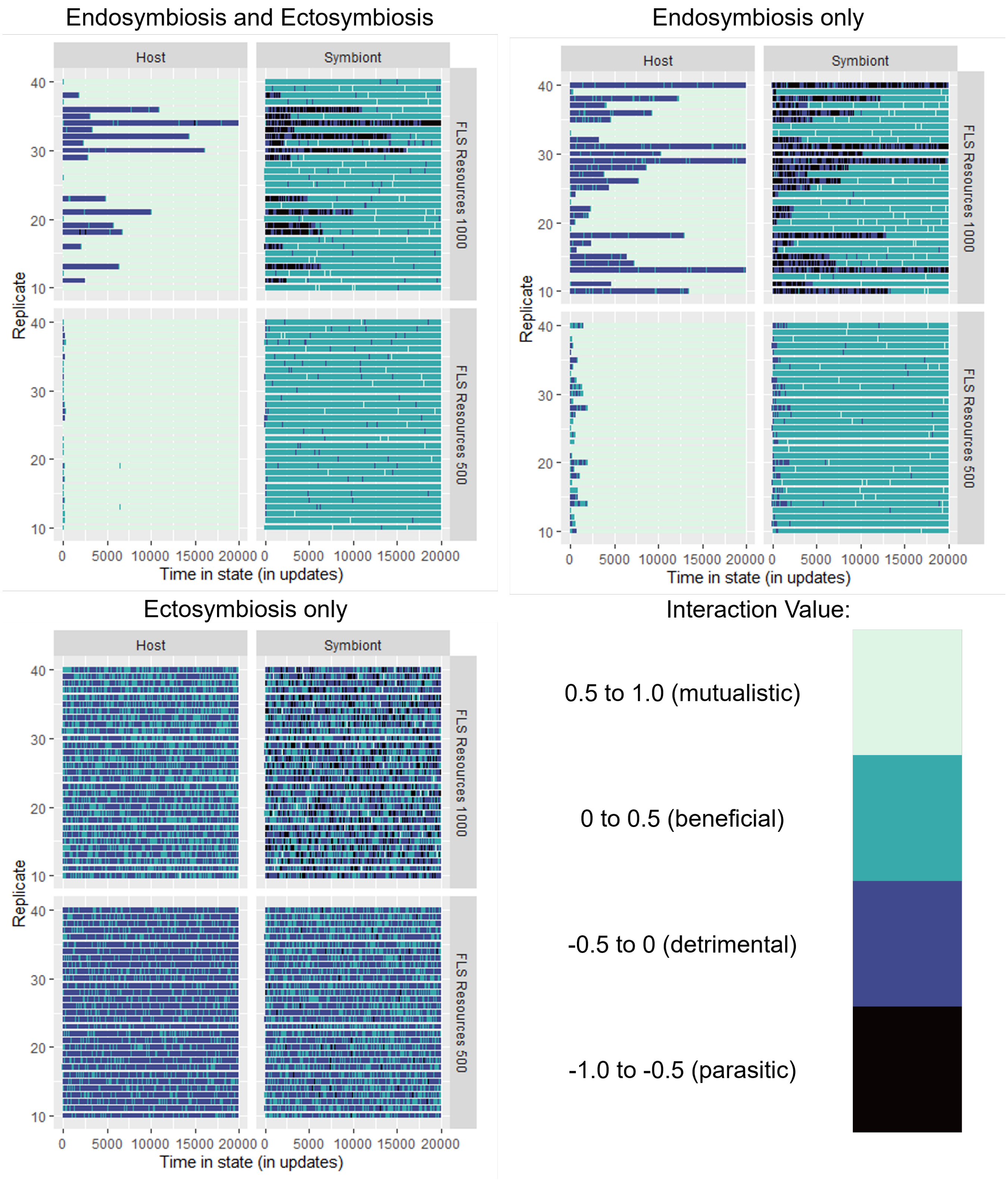

Figure 8: **Dominant lineage state sequences.** The dominant lineage is the sequence of ancestors (i.e. line of descent) of the most populous phenotype at the end of each experiment. Hosts were given 500 resources per update, and free living symbionts were distributed either 500 or 1000 resources. Interaction value categories are lower-bound inclusive, upper bound exclusive (except for the 0.5 to 1.0 state, which is 1.0-inclusive).

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