

# Evolution of symbiotic task-based digital genomes: ectosymbiosis hastens the evolution of endosymbiosis

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## Introduction

Endosymbiosis, in which a symbiont lives within its host, is a biological dynamic that has profoundly shaped evolution of life on earth: mitochondria are hypothesized to have originally been endosymbionts, providing the energy and genetic complexity necessary for the explosion of the eukaryotic clade and modern multicellular life (Lane and Martin, 2010). In humans, the gut microbiome (consisting of endosymbionts) plays a crucial role in digestion and overall health (Mohajeri et al., 2018). However, it is also a puzzling evolutionary dynamic: though endosymbionts can receive several benefits by living within hosts – protection from outside elements, resources, etc. – they also sacrifice a level of independence, occasionally to the extreme of depending upon the host for reproduction or life.

This conflict between benefits and costs raises the question of *what conditions drive the evolution of endosymbiosis, and in what situation does the diminished independence prove a more viable strategy?* Previous work has found that the presence of ectosymbiosis (in which organisms can interact but a symbiont does not live within a host) can increase rates of endosymbiosis in conditions where it is less likely to evolve alone, and depress it in conditions where it evolves strongly when alone (an overall equalizing effect) (Johnson et al., 2022). However, this past work used a high level of abstraction to model genomes, limiting the complexity of behavior that could evolve (Vostinar et al., 2021). Digital genomes implemented in this work permit a greater range of organism behaviors and more strategies for gaining resources and performing symbiotic actions. Consequently, this work’s findings are more applicable to systems with more complex behaviors. With these new methods, results indicate that endosymbiosis evolves more rapidly when ectosymbiotic behavior can evolve as well, in agreement with previous results.

## Methods

To investigate these dynamics, we integrated the SignalGP-Lite library, which provides genetic programming-based genomes (Moreno and Rodriguez Papa, 2022), with Symbu-

lation, an agent-based model of symbiotic evolution (Vostinar, 2021). In this new version of Symbulation, digital organisms have genomes made up of a specialized instruction set and must compete for limited space in a world. They gain resources by executing their instructions and completing logic tasks (similar to Avida’s ‘logic 9’ set, instruction set similar to Avida’s default, (Ofria and Wilke, 2004)); when they execute a ‘reproduce’ instruction, if they have enough resources, they are able to create an offspring. Offspring overwrite organisms in the cell that they are being born into, so speed of reproduction and rate of successful task completion are vital to survival and proliferation of a lineage. There are several complications to the completion of a task. Certain tasks can only be completed once every few updates per host (*task limits*) and other tasks depend on the completion of lower tasks first (*task dependencies*).

Symbionts and their hosts can interact in two ways: directly or through task dependencies. Both ectosymbionts and endosymbionts can directly donate resources to or steal resources from their host using specialized ‘donate’ and ‘steal’ instructions. They can also interact with hosts’ task limits and dependencies. For example, if the symbiont completes a limited task, the host cannot obtain resources by completing that task for the next few updates, leading to potential parasitism. Conversely, task dependency completions can be shared between host and symbiont, so the host can do a more advanced task if the symbiont is doing a simpler one and vice versa, providing a mechanism for mutualism. A host or endosymbiont can also choose to complete a task ‘privately’, where its partner can’t use its task completions to satisfy task dependencies, so organisms can evolve between parasitic and mutualistic interactions (note that completing a task ‘privately’ has a 20% penalty for hosts).

Symbionts can either occupy a host (endosymbiosis) or survive outside of a host (free-living) in a parallel population (they don’t compete for space with the host population). Free-living symbionts can evolve the ability to infect (via an instruction), at which point they can try to move into a geographically-proximate host. If the host already has a symbiont, the infecting symbiont will be killed upon the at-

tempt. If ectosymbiosis is permitted in the world, free-living symbionts can interact with a host in the parallel cell (in the host population) without infecting (meaning that if the host is killed by another host's offspring, the ectosymbiont does not die). Consequently, ectosymbiosis acts as a stepping-stone between total independence and endosymbiosis.

Symbionts can reproduce in three ways. First, free-living symbionts reproduce in the same manner as hosts; once they acquire sufficient resources they inject their offspring in their world, overwriting the organism in that location previously. Second, endosymbionts can be horizontally transmitted; the parent injects its offspring into the free-living population. Third, endosymbionts can reproduce by vertical transmission, in which they insert their offspring into the offspring of their host. Each of the three manners of reproduction have distinct resource thresholds, specified below.

To examine the influence of ectosymbiosis on the evolution of endosymbiosis, we examined the evolution of endosymbiosis when: 1) only endosymbiosis was permitted and 2) both ectosymbiosis and endosymbiosis were permitted. In each condition, we varied vertical transmission rate (0, 20, 40, 60, 80, and 100%) as well as resources required for endosymbiont horizontal transmission (250, 500, 750, 100, 1000; relative to 0 required for vertical transmission and 100 required for free symbiont reproduction). These factors have been previously shown to influence the evolution of endosymbiosis and degree of mutualism/parasitism (Johnson et al., 2022; Vostinar and Ofria, 2019), enabling investigation into the impact of the type of interaction on the evolution of endosymbiosis in this system. Each particular combination of endosymbiosis, ectosymbiosis, vertical transmission, and horizontal transmission threshold was replicated 30 times with different random number seeds.

Code, configuration settings, data, supplementary graphs, and analysis scripts are available at <https://github.com/K-Johnson-Horrigan/SGP-Endosymbiosis-Evolution>.

## Results and Discussion

We first investigated under what conditions endosymbiosis evolves at all. Figure 1 demonstrates that endosymbiosis evolves earlier when fewer resources are required for endosymbionts to horizontally transmit and the vertical transmission rate is 40%. All treatments began with no endosymbionts, so all observed endosymbionts evolve through infection of a host by a free living symbiont, which may or may not be an ectosymbiont. Specifically, the average count of endosymbionts in the world when 100 and 250 resources are required for horizontal reproduction are, respectively, 9206.5 and 645.8 at update 5000 when ectosymbiosis is not permitted (Wilcoxon rank sum test,  $p < 3.018e - 11$ ). This result is likely because the endosymbionts in the lower horizontal transmission resource threshold condition can reproduce more quickly and spread their genotypes (which

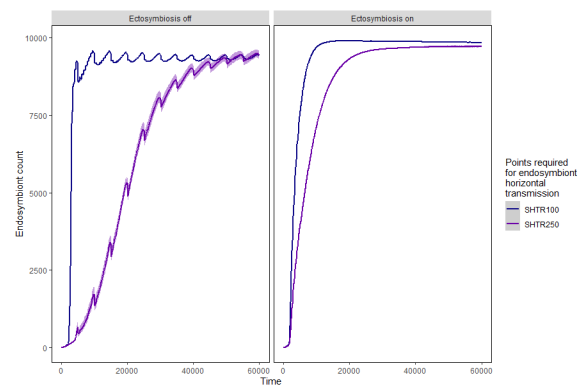


Figure 1: Average count of endosymbionts over time with and without ectosymbiosis permitted and when resources required for horizontal transmission was 100 and 250. Vertical transmission rate fixed at 40%.

include the instruction for infecting hosts) more rapidly through the symbiont population.

Endosymbiosis also evolves more rapidly when ectosymbiosis is permitted (mean endosymbiont count at 5000 updates when 250 resources are required for horizontal transmission with and without ectosymbiosis is 4000.833 and 645.8, respectively; Wilcoxon rank sum test with continuity correction,  $p < 3.018e - 11$ ). This result could occur because ectosymbionts reproduce – and thus evolve – more rapidly than non-ectosymbiotic free living symbionts.

Finally, we also investigated how ectosymbiosis affects the evolution of mutualistic/parasitic endosymbiosis. We defined parasitism as a relationship where the resources stolen:donated ratio was higher than 1. 294 replicates out of 300 are parasitic by this definition (data in supplemental material). Almost all replicates when ectosymbiosis is not permitted produce higher final rates of mutualism than when ectosymbiosis is permitted (data in supplemental material, mean stolen:donated resource ratio at the end of replicates with and without ectosymbiosis when 1000 resources are required for horizontal transmission is 2.833578 and 1.316859, respectively; Wilcoxon rank sum test with continuity correction,  $p < 2.2e - 16$ ). When ectosymbiosis is not permitted, overall symbiont populations get less parasitic as the resources required for endosymbiont horizontal transmission increase (mean stolen:donated resource ratio without ectosymbiosis at 100 and 1000 resources required for horizontal transmission is 2.171647 and 1.316859, respectively). Wilcoxon rank sum test with continuity correction,  $p < 2.2e - 16$ ). Generally, mutualistic endosymbiosis evolves most often when ectosymbiosis is not permitted, vertical transmission rate is high, and the number of resources required for horizontal endosymbiont transmission is also high.

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