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Characterizing the evolution of defense in a tripartite marine symbiosis using adaptive dynamics

Prerna Singh[1] ID, Marjolein Bruijning[2], Gavriela D. Carver[3], Mohamed S. Donia[1,3], Charlotte Jessica E. Metcalf[1,] ID

[1]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, United States
[2]Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands
[3]Department of Molecular Biology, Princeton University, Princeton, NJ, United States

Corresponding author: Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, United States. Email: cmetcalf@princeton.edu

Abstract

The evolution and maintenance of symbiotic systems remains a fascinating puzzle. While the coevolutionary dynamics of bipartite (host–symbiont) systems are well-studied, the dynamics of more complex systems have only recently garnered attention with increasing technological advances. We model a tripartite system inspired by the marine symbiotic relationship between the alga *Bryopsis* sp., its intracellular defensive bacterial symbiont "*Candidatus* Endobryopsis kahalalidifaciens," which produces a toxin that protects the alga against fish herbivores, and the sea-slug *Elysia rufescens* (Zan et al., 2019), which is not deterred by the toxin. We disentangle the role of selection on different actors within this system by investigating evolutionary scenarios where defense evolves as (i) a host-controlled trait that reduces algal reproductive ability; (ii) a symbiont-controlled trait that impacts symbiont transmission; and (iii) a trait jointly controlled by both host and symbiont. Optimal investment in defensive toxins varies based on the characteristics of the host, symbiont, and sea slug; and evolutionary trajectories are modulated by trade-off shape, i.e., a strongly decelerating trade-off between defense and symbiont transmission can drive symbiont diversification via evolutionary branching. Increasing slug herbivory reduces host investment in defense to favor reproduction, while symbiont investment in defense first declines and then increases as host density declines to the degree that horizontal symbiont transmission is no longer beneficial. Increasing vertical transmission selects for reduced defense by the host when it evolves as a jointly controlled trait, as a result of investment by the symbiont. Our theoretical exploration of the evolution of defensive symbiosis in scenarios involving interactions with multiple herbivores provides a first window into the origin and maintenance of the *Bryopsis* sp. system, and adds another piece to the puzzle of the evolution of symbiotic systems.

Keywords: endosymbiont, adaptive dynamics, trade-off, algae, kahalalides

Lay Summary

This study delves into a tripartite marine symbiosis between the algal host *Bryopsis* sp., and its protective bacterium Ca. *Endobryopsis kahalalidifaciens*, and the sea slug *Elysia rufescens* which feeds on bacterium-carrying algae. The bacterium produces defensive toxins that protect the algae from a range of herbivores. We developed a host–symbiont–herbivore model to study how this defensive relationship evolves in the presence of sea slugs which are not deterred by the toxins. We assumed that producing more toxins for the algal host means fewer resources for its reproduction, while for the bacterium, it means reduced ability to spread to new hosts. We discovered that the alga and bacterium's investment in toxin production is influenced by the strength of such defense costs, the magnitude of herbivory by sea slugs and other environmental factors. As such, when producing toxins becomes too costly, both alga and bacterium reduce their investment in defense. However, under certain conditions, multiple bacterium populations that have different levels of defense can coexist. This research highlights the evolution and maintenance of complex symbiotic relationships, offering insights into how organisms work together to survive in the face of multiple threats.

Across the tree of life, eukaryotes have repeatedly formed symbiotic relationships with prokaryotes (Smith & Szathmary, 1997). Organelles like mitochondria and chloroplasts, central to energy generation across metazoans, reflect an extreme of this relationship: originated from free-living bacteria and entered into an obligate symbiotic relationship with ancestral eukaryotic cells (the relationship is described as an "endosymbiosis"). Beyond these extreme examples, there is a tremendous diversity in the scale of depende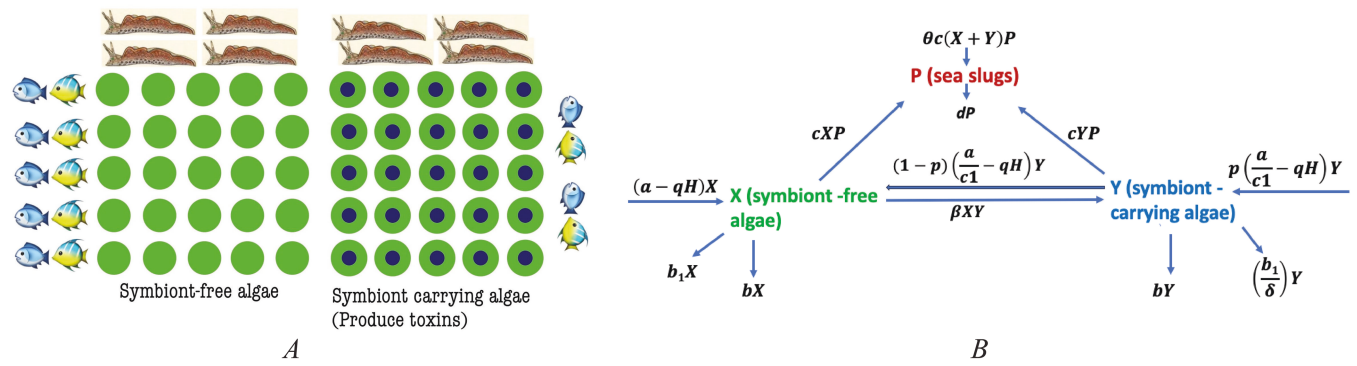nce and integration of symbiotic prokaryotes, from facultative symbionts of pea aphids that supply them with essential amino acids obtained from the host plant (Akman Gu̇ndu̇z & Douglas, 2009) to bioluminescent bacterial partners that support camouflage of the bobtail squid (Visick et al., 2021), and more examples are regularly added (Burghardt, 2020; Ford et al., 2022; Koskella & Brockhurst, 2014; Rafaluk-Mohr et al., 2018; Song et al., 2021). While there is significant literature on symbiosis formation, ranging from host exploitation (Law & Dieckmann, 1998) to the evolution of reduced horizontal transmission in symbionts

Figure 1. (A) Illustration showing two algal populations, one with symbiont and one without, encountering herbivory from sea slugs (top) and general herbivores like fish (left and right). Symbiont-carrying algae, producing defensive toxins, are less susceptible to general herbivory, while sea slugs consume both algal types equally. (B) Model schematic diagram. Arrows indicate transitions in or out of respective states at the indicated rates.

(Nguyen & van Baalen, 2020), gaps remain in understanding which evolutionary features promote symbiosis in specific systems. Additionally, fitness trade-offs can significantly influence the origin of endosymbiosis, driving the selective processes shaping its early evolution (Brockhurst et al., 2024). The extensive theory also exists on symbiont roles in host defense against parasites (Ashby & King, 2017; Fenton et al., 2011; Haine, 2008; Jones et al., 2011; Kwiatkowski & Vorburger, 2012; Rafaluk-Mohr et al., 2018; Smith & Ashby, 2023), but less is known about symbiont-conferred defenses against herbivores despite the empirical evidence (Tianero et al., 2019; Zan et al., 2019). In particular, how host–symbiont associations thrive in the presence of multiple herbivore (or predator) species and trade-offs have yet to be explored theoretically.

The intracellular defensive bacterial symbiont "*Candidatus* Endobryopsis kahalalidifaciens," found exclusively in the alga *Bryopsis* sp., represents a unique opportunity to probe these questions. In this system, collaborative biosynthesis between the bacterium and its algal host results in the production of a diverse range of chemical defense toxins (the kahalalides) (Zan et al., 2019), which deter a range of herbivores including many fish species from grazing on the alga (Becerro et al., 2001). For the alga, the fitness benefit of reduced mortality from diminished grazing is likely to offset the energetic costs of bacterial carriage (Vorburger & Gouskov, 2011; Vorburger & Perlman, 2018; Zan et al., 2019). For the bacterium, while benefits likely reflect a relatively stable growth environment, the costs of symbiosis are harder to identify, as the origin of the bacterium/alga association remains largely enigmatic. One potential cost is genome reduction, which makes the bacteria completely reliant on the host. Host-to-host transmission (horizontal transmission) is a common method for microbial acquisition in marine environments (Roughgarden, 2023); yet in this system, the bacterium's apparent loss of capacity for persistence outside of the host suggests that vertical transmission might be more likely. Importantly, for both parties, the benefit of the symbiosis is mitigated by another unique feature of this system: the existence of a kleptoplastic sea-slug: *Elysia rufescens* is resistant to the toxins and capable of sequestering them from its algal diet for its own protection from various predators (Hamann & Scheuer, 1993; Mascuch & Kubanek, 2019; Zan et al., 2019), making algae a specialized source of food for this species. However, it is not yet proven whether the slugs preferentially eat algae that carry the symbiont compared to those that do not, although this is an interesting direction for future empirical work. Consequently, the algae experience pressure from two

types of herbivores: sea slugs, unaffected by defensive toxins, and general herbivores like fish, which are deterred by these toxins and thus have reduced ability to consume algae carrying defensive bacteria (see Figure 1).

To evaluate how this unique symbiosis persists, even in the presence of the sea slug that can erode its value, we develop a tripartite mathematical model encompassing the algal host, the mutualistic symbiont acquired through mixed mode of transmission (horizontal and vertical), and the slug herbivore. We comprehensively explore the life history and ecological features that shape the evolution of defense against general herbivory. We root our analysis around trade-offs affecting fitness for the host and algal symbiont separately: reduced algal reproduction with increased resource allocation towards toxin production for the host; and reduced horizontal symbiont transmission with increased toxin production for the symbiont. Empirical evidence for the latter is scant, but it is reasonable to assume that the bacterium must have originally had alternative forms of transmission that importantly contributed to fitness (Smith & Ashby, 2023) to which allocation was diminished with increased allocation towards toxin production. Existing theoretical models on host–symbiont evolution have addressed aspects such as the evolution of symbiont-conferred defense against parasites (Ashby & King, 2017; Fenton et al., 2011; Kwiatkowski & Vorburger, 2012), coevolution of symbionts with parasite virulence (Jones et al., 2011; Rafaluk-Mohr et al., 2018; Smith & Ashby, 2023), or host–parasite evolution in the presence of symbionts (King & Bonsall, 2017). However, none of these studies has investigated how host immune responses and symbiont-conferred defense evolve in response to herbivore attacks, despite clear empirical evidence for this (Morita & Schmidt, 2018; Paul et al., 2007). Therefore, in addition to providing a theoretical framework for this algae-bacterium-slug symbiosis, our model also highlights understudied aspects of defensive symbiosis evolution in response to multiple herbivores or predators.

With our framework in hand, we address a series of biological questions associated with this tripartite interaction, specifically: (i) the magnitude of algal host and bacterial symbiont allocation toward toxin production given associated fitness costs (i.e., algal reproduction and symbiont transmission, respectively); (ii) how is this modulated by the presence of sea slugs or other host life history features, including vertical transmission and density-dependent crowding, and (iii) how these investments vary when algae and symbiont coevolve, compared to when they evolve separately. Our analysis delineates the adaptive strategies that shape the selection for defense against general herbivory, despite the

tradeoffs it requires from both host and bacterial species and the existence of the sea slug whose existence reduces the benefit of toxin production.

The Model

Host evolution model: defense against general herbivory with cost to reproduction

To explore the evolution of symbiosis in this system requires a model encompassing the population of algal hosts, that may be either symbiont-free (denoted by X) or carrying the defensive symbiont (denoted by Y); and that face herbivory from sea slugs (denoted by P) and are not sensitive to the toxin produced by the symbiont; and other general herbivores such as fish (population dynamics not explicitly modeled). We assume that there is no free-living stage of the symbiotic bacterium, and its population dynamics are reflected by Y. The following equations illustrate the population dynamics of the model:

$$\frac{dX}{dt} = (a - qH)X + (1-p)\left(\frac{a}{c_1} - qH\right)Y - \beta XY - bX - cXP - bX,$$

$$\frac{dY}{dt} = \beta XY + p\left(\frac{a}{c_1} - qH\right)Y - bY - cYP - (b_1/\delta)Y,$$

$$\frac{dP}{dt} = \theta c(X + Y)P - dP. \tag{1}$$

Here, $H = X + Y$ and the parameters are detailed in Table (1). Algal hosts H have an overall birth rate a, natural death rate b, and q is the impact of density-dependent crowding acting on the host birth. We assume that the algal host can acquire the bacterial symbiont either horizontally or vertically. The parameter p reflects the degree of vertical transmission ($0 \le p \le 1$): a proportion p of offspring from hosts in state Y inherit the symbiont, while the remaining are born symbiont-free. The parameter β indicates horizontal transmission, via a mass-action contact process between two algal populations. The reproductive rate of hosts carrying the symbiont is reduced to a/c_1 to reflect resources allocated to sustaining the bacterial symbiont. We assume that general herbivores such as fish consume the algal host at a constant rate, b_1, implying that any feedback from the algal system on the herbivore populations is negligible; and define δ as the magnitude of symbiont-conferred protection against such herbivores. The larger the magnitude of δ, the less general herbivores are able to feed on symbiont-carrying hosts ($\delta \ge 1$). Practically, δ might reflect the amount of toxic lipopeptide molecule kahalalide F released by the intracellular bacterial symbiont. Unlike

Table 1. Description of parameters.

Parameters	Definition	Default value
a	Host birth rate	3.5
b	Host natural death rate	0.1
q	Crowding effect	0.1
p	Vertical transmission of the symbiont	0.5
β	Horizontal transmission of the symbiont	2
d	Slug natural death rate	0.1
δ	Defense against general herbivores	Varies
c	Slug herbivory rate	0.3
c_1	Cost to sustain the symbiont	1.2
b_1	Deaths due to general herbivores	3
θ	Conversion of herbivory into slug reproduction	0.3

other herbivores, the slug (whose dynamics are captured by P, and which dies at rate d), is not repelled by the toxin, and can even use it for its own defense, i.e., the algal host has no defenses against this herbivore, whether carrying the symbiont or not. Given the lack of empirical evidence, we assume that the slug cannot distinguish between symbiont-free or symbiont-carrying alga, and consume both algal host populations at a rate of consumption c.

We choose our parameters such that all population densities coexist at an endemic equilibrium (X^*, Y^*, P^*). These values ensure biological plausibility with a focus on the theoretical insights provided by model dynamics. While deriving them experimentally could be possible, it is outside the scope of this study. We explore how defense against general herbivores evolves within various trade-off frameworks. Initially, we investigate defense as a host-controlled (algae-controlled) trait, where the host regulates the production of defensive toxins via amino acid resource allocation, at the expense of its reproductive capacity—a scenario indicative of an algal reproduction-defense trade-off. Subsequently, we examine defense as a symbiont-controlled trait, wherein the symbiont governs toxin production at the expense of reduced transmission ability. In both scenarios, we analyze potential evolutionary outcomes based on trade-off shapes and ascertain how stable defense investment fluctuates based on factors influenced by the fitness of the host, symbiont, and herbivore. We also examine how both host and symbiont jointly control defense in coevolution and outline the differences observed between evolutionary and coevolutionary frameworks.

Methods

We use adaptive dynamics theory (Geritz et al., 1997; Metz et al., 1992, 1995) to study the evolution of defense against general herbivores when higher defense leads to a reduction in algal host reproduction (host evolution), and when it leads to lower symbiont transmission (symbiont evolution). This method involves introducing a rare mutant "invader" in a system that is at its ecological equilibrium, with a slightly different phenotypic value of the adaptive trait relative to a "resident" strategy. We map the success or failure of repeated invasions to understand how the trait evolves over time (Geritz et al., 1998). Our evolutionary framework is general and can be applied to similar tripartite systems. When considering defense as a host-controlled trait (i.e., host evolution), we assume that a mutant host strain with strategy (δ_m, a_m) arises and potentially invades the resident host strain with strategy (δ, a), where the latter is currently at its stable equilibrium. The invasion fitness of the mutant host is sign equivalent to the negative determinant of the Jacobian matrix of mutant dynamics system and is given by:

$$s_1(\delta_m, \delta) = -(a_m - qH^* - \beta Y^* - b - b_1 - cP^*)$$
$$\left(p\left(\frac{a_m}{c_1} - qH^*\right) - b - cP^* - \frac{b_1}{\delta_m}\right)$$
$$+ \beta Y^*(1-p)\left(\frac{a_m}{c_1} - qH^*\right) \tag{2}$$

if the parameters are chosen such that $p\left(\frac{a_m}{c_1} - qH^*\right) < b + cP^* + \frac{b_1}{\delta_m}$ (see Hoyle et al. (2012) and Supplementary File for more details). This expression works as a proxy for the mutant fitness (Miller et al., 2006), where $s_1(\delta_m, \delta) > 0$ means that the mutant strain can spread and invade the resident strain. Using evolutionary invasion analysis (Geritz et al., 1998), we can determine the position of evolutionary singular points (where the fitness gradient $\frac{\partial s_1(\delta, \delta_m)}{\partial \delta_m}$ becomes zero) and the evolutionary behavior at these points. The

evolutionary outcome is determined by two stability conditions: evolutionary stability (ES), where $\frac{\partial^2 s_1}{\partial \delta_m^2}|_{\delta=\delta_m=\delta^*} < 0$, and convergence stability (CS), where $\frac{\partial^2 s_1}{\partial \delta_m^2} + \frac{\partial^2 s_1}{\partial \delta \partial \delta_m}|_{\delta=\delta_m=\delta^*} < 0$.

Evolutionary stability assesses whether further mutations can invade a strategy, and convergence stability evaluates if the strategy is evolutionarily attractive. A singular point possessing both evolutionary and convergence stability is termed a continuously stable strategy (CSS) (Eshel, 1983). Initially, we explore how CSS varies with other ecological factors, i.e., we only consider the "singular points" in the trait space that exhibit both types of stability, thus serving as long-term attractors of evolution (Geritz et al., 1998).

Higher investment in the production of defensive compounds requires the provisioning of resources to the symbiont which might reduce resources available for algal reproduction, leading to a trade-off between algal reproduction and defense against general herbivory. Based on this hypothesis, we consider a generic trade-off function relating the parameters reflecting these traits: a and δ respectively (see Hoyle et al. (2012) for more details on the trade-off form) as follows:

$$a(\delta) = a(\delta^*) - \frac{a'(\delta^*)^2}{a''(\delta^*)}\left(1 - e^{\frac{a''(\delta^*)(\delta-\delta^*)}{a'(\delta^*)}}\right). \quad (3)$$

Here, $a'(\delta^*)$ and $a''(\delta^*)$ represent the slope and curvature of the trade-off curve, respectively. Adopting this trade-off formulation enables us to fix the singular strategy $(\delta^*, a(\delta))$ at a designated point and subsequently calculate the slope and curvature of the trade-off function at this chosen strategy.

The slope is determined such that the singularity occurs at chosen points i.e., the fitness gradient $\frac{\partial s_1}{\partial \delta}$ becomes zero at δ^*. Conversely, the curvature dictates the trade-off's shape and can be tailored to achieve an accelerating or decelerating trade-off. In this section, we use an accelerating trade-off function (Figure 2), which fosters stable investments in the protection trait δ^* and thus corresponds to a CSS. Biologically, such a trade-off shape implies that the cost of providing protection against general herbivores progressively rises.
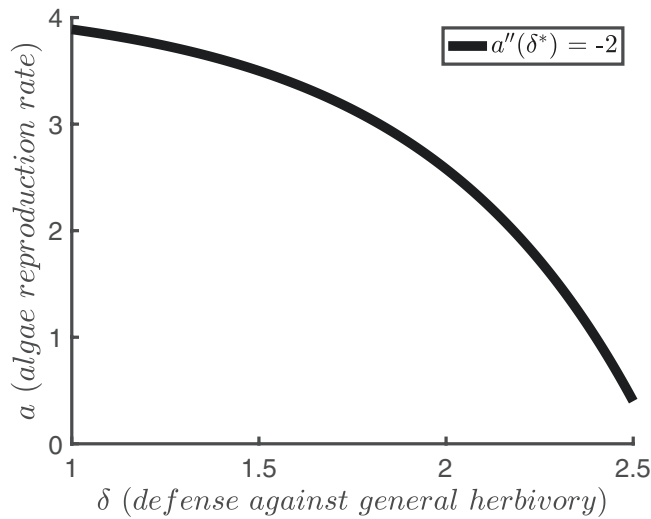
Figure 2. An accelerating trade-off function curve showing that the increasing defense against general herbivores leads to lower algal reproduction. Here, the slope is a'(δ') = −1.16128 for the singular strategy (δ*, a(δ)) = (1.5, 3.5).

Continuously stable strategies representing algal host defense

We explore the influence of parameters reflecting host characteristics (via density-dependent crowding, q), symbiont characteristics (via the degree of vertical transmission of the symbiotic bacterium, p), and sea-slug characteristics (via the rate of herbivory, c) on the evolution of host defense against general herbivores by mapping variation in the CSS investment in defense, δ (Figure 3A–C). Increasing

density-dependent crowding within the host population negatively impacts host reproduction capacity by introducing higher competition for resources. As the fitness contribution of reproduction is reduced, the fitness contribution of host survival via defense against general herbivores increases in importance, driving a marginal monotonic increment in the CSS levels of defense, as crowding q increases (Figure 3A). Increasing vertical transmission of the symbiont also selects for higher investment in defense against general herbivores δ (Figure 3B), since the larger the fraction of offspring that has the symbiont, the greater the returns on investment in defense via the symbiont. Conversely, greater intensity of sea slug herbivory, c, reduces CSS levels of defense, since this defense is ineffectual against the slug, and thus there are greater fitness returns from the investment in host reproduction instead (Figure 3C). It is important to note that these investment patterns are driven as per the fitness costs and benefits associated with specific ecological conditions.

Symbiont evolution model: defense against general herbivory with cost to transmission

Next, we analyze defense as a symbiont-controlled trait. We assume that the cost to the bacteria of investment in defense is reduced potential for horizontal transmission. Although such a trade-off has not been empirically characterized, investment in defensive toxin compounds is costly to the symbiont: the result might be a number of life-history trade-offs, but a reduction in transmission ability has clear fitness consequences. We frame the tradeoff relating defense δ and horizontal transmission β similarly to the above (eq. 3). The invasion fitness expression of the symbiont-carrying mutant strain with strategy (δ_m, β_m), which attempts to invade the resident strain with strategy (δ, β) fixed at its equilibrium is given by:

$$r(\delta_m, \delta) = \beta(\delta_m) X^* + p\left(\frac{a}{c_1} - qH\right) - b - cP^* - \frac{b_1}{\delta_m}. \quad (4)$$

Continuously stable strategies representing symbiont-conferred defense

Assuming that the investment in increasing defense becomes increasingly costly in terms of lower transmission (accelerating trade-off), we repeat the analysis for CSS investment in defense for the same ecological parameters (crowding, vertical transmission, and slug herbivory rates) in Figure 4. Increasing crowding q drives a similar monotonic increment increase in defense δ for similar reasons (larger returns on investment in survival under reduced reproduction) but the magnitude of the increment is larger when defense is a symbiont-determined trait rather than a host-determined trait (Figure 4A, compare with Figure 3A). Increasing vertical transmission also selects for a monotonic increase in the CSS levels of investment in defense (Figure 4B), again as a result of increased returns on investment via offspring defense. Moreover, when the cost of high defense is lower transmission, a high degree of vertical transmission balances out the transmission loss due to defense and can thus make defense less costly, driving selection

for increased defense. Interestingly, the CSS investment in defense is *U*-shaped across magnitudes of sea slug herbivory, *c*, and minimal for intermediate levels (Figure 4C). As the pressure of sea-slug herbivory increases, the returns on general herbivore defense are reduced, and the CSS investment in defense accordingly declines. Beyond a threshold, however, sea-slug herbivory is of such large magnitude that the total host population is much reduced, which in turn diminishes returns on horizontal transmission, and makes investment in defense against other herbivores necessary to ensure host (and thus symbiont) survival.

### Strongly decelerating costs can lead to polymorphic symbiont strains

Continuously stable strategies are not the only possible evolutionary outcome in this system: when costs are strongly decelerating (i.e., there is a relatively large initial cost to transmission but the additional costs become less extreme at higher protection levels, see Figure 5A), evolutionary branching can occur (Geritz et al., 1998), which can ultimately result in the coexistence of multiple symbiont strains with different scales of investment in defense within the population. In particular, for a range of trade-off curvatures $\beta''(\delta)$ ranging from approximately 3 to 6.2 for our default parameter set, branching can arise (see Supplementary File). Even though the specific range may shift with parameter changes, the core finding that strongly decelerating trade-off shapes drive branching would remain consistent. Interestingly, this outcome is only observed at the scale of symbiont evolution: extensive

analytical exploration indicates that a trade-off between host reproduction and defense never leads to branching.

The pairwise invasibility plot (Geritz et al., 1997; Metz et al., 1995) for this scenario is provided in Figure 5B: black regions indicate areas of invasion success by the symbiont-carrying mutant strain, and white areas indicate where the invasion is not possible. The singular strategy, which is a branching point (i.e., mutants with higher or lower defense levels can invade the resident) lies at the intersection of these two regions. Symbiont populations are expected to evolve toward the branching point, but disruptive selection at the branching point leads to the occurrence of coexisting symbiont-carrying host strains: one is highly defended against general herbivores (but has lower transmission ability), and another is less defended (but highly transmissible). The strain conferring minimal defense would be similar to a parasitic strain, as the host is still investing resources towards carrying the bacteria. Analytical analysis confirms the existence of branching, i.e., ES = $\frac{\partial^2 r}{\partial \delta_m^2}|_{\delta=\delta_m=\delta^*} > 0$, and CS = $\frac{\partial^2 r}{\partial \delta_m^2} + \frac{\partial^2 r}{\partial \delta \partial \delta_m}|_{\delta=\delta_m=\delta^*} < 0$ at the singular strategy 1.5 (see Supplementary File).

## Coevolution: host and symbiont jointly control defense

So far, we evaluated host and symbiont scale selection for defense against general herbivores separately. In reality, both could coevolve and jointly control the evolution of defense. In the algal-symbiotic bacterium system, the bacterium relies on the alga for the metabolic building blocks required for kahalalide (toxins)
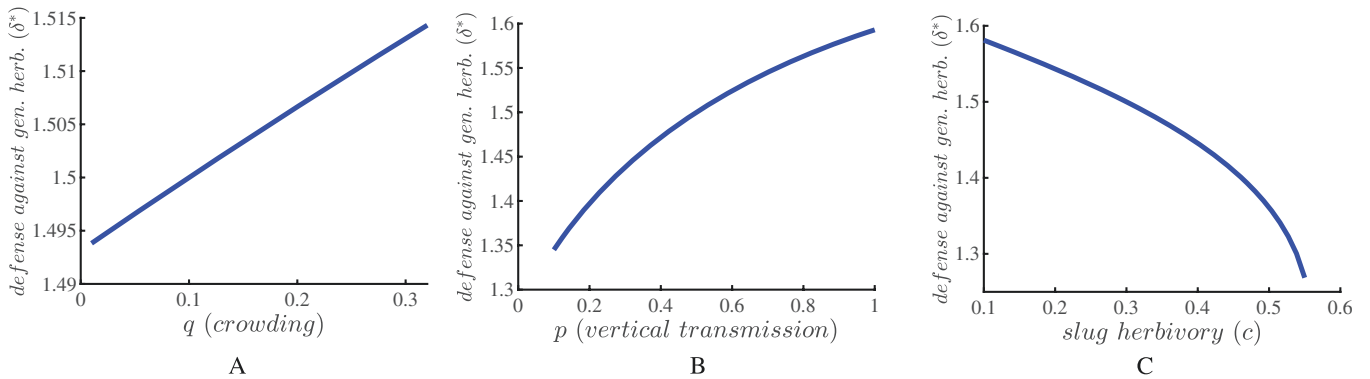
Figure 3. CSS investment variation in host defense δ corresponding to increasing (A) density dependent-crowding *q*, (B) vertical transmission *p*, and (C) slug herbivory rate *c*. Assuming the defense-reproduction trade-off, we use $a'(\delta) = -1.16128$, $a''(\delta) = -2$, and the remaining parameters are the same as in Table 1.
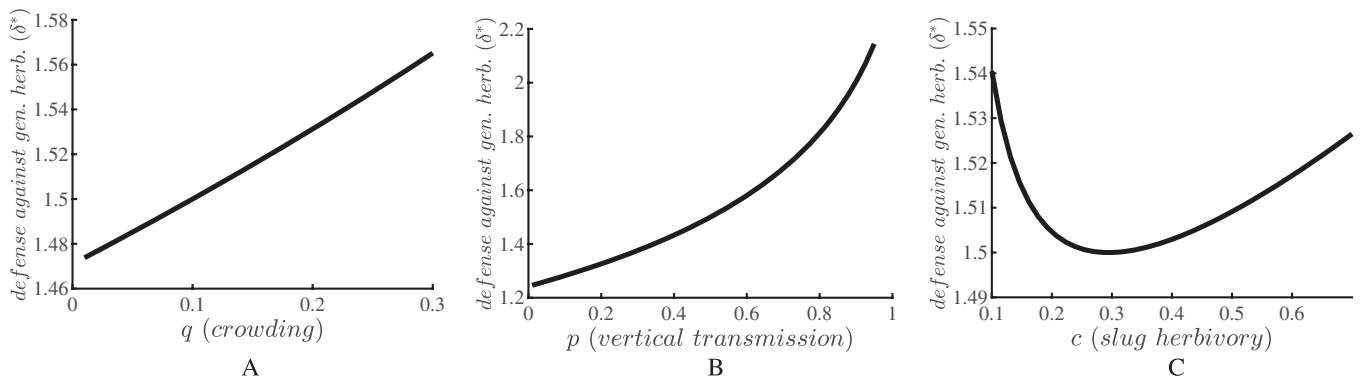
Figure 4. (A–C) CSS investment variation in symbiont-conferred defense δ corresponding to increasing density-dependent crowding *q*, vertical transmission *p*, and slug herbivory rate *c*. Assuming the defense-transmission trade-off, we use $\beta'(\delta) = -2.165$, $\beta''(\delta) = -1.5$, and remaining parameters are as in Table 1.
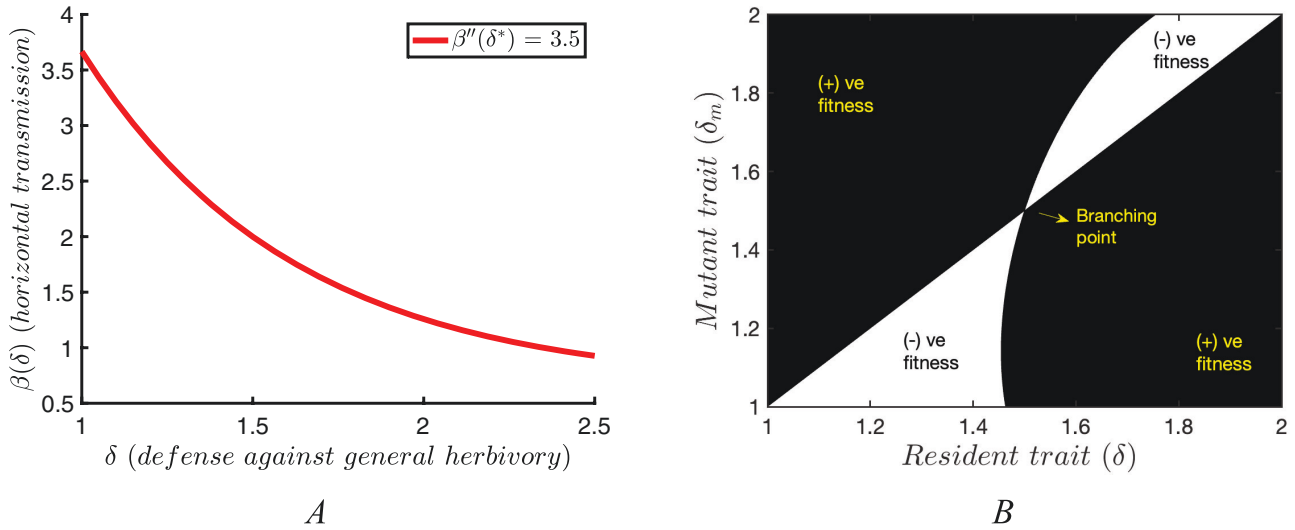
Figure 5. (A) Transmission-defense trade-off curve with decelerating costs. (B) PIP shows the occurrence of a branching point at 1.5 for this trade-off shape.

production. In return, the bacterium provides transcriptional activity to produce these chemicals (Mascuch & Kubanek, 2019), suggesting joint control of the production of defensive toxins. To address this, we allow both host and the symbiont populations to simultaneously evolve their respective share of the defense trait through small mutational events. For both, increasing defense against general herbivory is costly elsewhere in their life history, as previously described, reducing host reproduction $a(h)$ and symbiont horizontal transmission $\beta(s)$, respectively. Furthermore, the defense trait δ is defined via a combination of host investment in defense h, and symbiont investment in defense, s, with $\delta(h, s) = hs$. Such a function has been widely applied in theoretical studies to describe how two species exert control over a trait (Best, 2018; Best et al., 2014; Restif & Koella, 2003). Incorporating these assumptions, the model is given by:

$$\frac{dX}{dt} = (a(h) - qH)X + (1-p)\left(\frac{a(h)}{c_1} - qH\right)Y$$
$$- \beta(s)XY - bX - cXP - b_1X,$$
$$\frac{dY}{dt} = \beta(s)XY + p\left(\frac{a(h)}{c_1} - qH\right)Y - bY - cYP$$
$$- \frac{b_1}{\delta(h,s)}Y,$$
$$\frac{dP}{dt} = \theta c(X+Y)P - dP. \tag{5}$$

In this coevolutionary framework, we model defense control by considering the emergence of a mutant strain characterized by the strategy (h_m, s_m) attempting to invade the resident equilibrium. The invasion success depends on the sign of the invasion fitness for both species involved. To quantify this, we use the following fitness proxy expressions for the host and symbiont, respectively:

$$s_1(h, h_m, s) = -(a(h_m) - qH^* - \beta(s)Y^* - b - b_1 - cP^*)$$
$$\left(p\left(\frac{a(h_m)}{c_1} - qH^*\right) - b - cP^* - \frac{b_1}{\delta(h_m, s)}\right)$$
$$+ \beta(s)Y^*(1-p)\left(\frac{a(h_m)}{c_1} - qH^*\right),$$
$$r(h, s, s_m) = \beta(s_m)X^* + p\left(\frac{a(h)}{c_1} - qH\right) - b - cP^* - \frac{b_1}{\delta(h, s_m)}, \tag{6}$$

where the subscript m indicates the mutant trait and the condition $p\left(\frac{a(h_m)}{c_1} - qH^*\right) < b + cP^* + \frac{b_1}{\delta(h_m, s)}$ holds. The host and symbiont populations then coevolve along their respective fitness gradients, $(\partial s_1/\partial h_m)\,|_{h_m=h}$ and $(\partial r/\partial s_m)\,|_{s_m=s}$, forming a

coevolutionary trajectory until a cosingular point (h,s) is attained where the two gradients become simultaneously zero (Durinx et al., 2008). For a cosingular point to be a convergent stable strategy (i.e., a co-CSS), it must satisfy the evolutionary stability and convergent stability conditions, derived from the second-order derivatives of host and symbiont fitness expressions (see Supplementary File).

Next, we show how the CSS investments in defense against general herbivory differ in the coevolutionary scenario as compared to when the host and symbiont evolved in isolation with their respective costs to reproduction and transmission (Figure 6). Defense investments under evolution (solid lines) and coevolution (dotted lines) are both shown for ease of comparison. Although the effects of density-dependent crowding and slug herbivory rates on defense investments remain similar in coevolution and separate evolution scenarios (see Supplementary File), the degree of vertical transmission of the symbiont creates distinct feedback for the host in the coevolutionary context, while the symbiont's investment remains consistent across both states (Figure 6). Initially, higher vertical transmission selects for increased host defense in both evolutionary scenarios. However, when transmission becomes sufficiently high, coevolutionary dynamics lead to a decline in the host defense, forming a subtle downward U-shaped pattern (dotted blue curve). In contrast, host defense continues to increase alongside transmission when it evolves in isolation (solid blue curve). When both the host and symbiont coevolve defense, high degrees of vertical transmission select for higher symbiont-conferred defense. This adaptation reduces the need for the host to invest heavily in its own defense mechanisms. Instead, the host can enhance its fitness by diverting its resources towards reproduction. This selective pressure is absent when only the host evolves defense, leading to divergent evolutionary trajectories.

Finally, we plot the variation in CSS levels of defense under the combined effects of both types of herbivories illustrating levels of protection when the cost of defense is at the scale of the host, e.g., lower algal reproduction (Figure 7A), and when the cost of defense is at the scale of the symbiont, e.g., reduced horizontal transmission (Figure 7B), and when both can share control over defense (Figure 7C). In all cases, in line with intuition, heightened pressure from general herbivores, indicated by high b_1, drives increased investment in defense against them.

Likewise, high magnitudes of slug herbivory alongside lower general herbivory select for low defense levels (blues hues) as higher investment in algal reproduction or symbiont transmission yields greater benefits than investing resources to counter rare general herbivores and ineffectual protection against widespread slugs. Sufficiently high general herbivory results in the nonmonotonic relationship described in Figure 4C, yielding the pattern shown for transmission-defense trade-off (Figure 7B). When both host and the symbiont share control over defense evolution along with the associated costs to reproduction and transmission respectively, the magnitudes of general herbivory exert even stronger control over defense as compared to when either species evolves separately (Figure 7C). These findings highlight the role of general herbivory in this tripartite symbiosis, suggesting that general herbivores might be another key driving force in the evolution of defensive toxins.

Discussion

Our mathematical model explores how an algal host and its bacterial symbiont evolve defensive toxins against general herbivores,

Figure 6. Plot showing how defense against general herbivory (δ) in coevolution (co-CSS) is different to when host and symbiont evolve separately, corresponding to varying vertical transmission p. Accelerating trade-offs are used, where $a'(\delta^*) = -1.16$, $a''(\delta^*) = -2$, $\beta'(\delta^*) = -2.165$, and $\beta''(\delta^*) = -1.5$. The remaining parameters are the same as in Table 1.

in the presence of a sea slug which remains unaffected by these toxins. Our key findings were: (i) increasing host crowding and vertical transmission of the symbiont selects for higher investment in defense, irrespective of whether the cost is to algal reproduction or the symbiont's transmission; (ii) increased slug herbivory selects for reduced defense against other herbivores if the cost is to reproduction, but defense follows a *U*-shaped curve for a symbiont transmission-defense trade-off, with lowest investments in defense at intermediate slug herbivory rates; (iii) a strongly decelerating trade-off between transmission and defense can trigger evolutionary branching in the symbiont-carrying algal population, producing two coexisting symbiont strains, one investing highly in defense, and one approaching a parasitic life style; (iv) when both host and symbiont coevolve defense with costs to reproduction/transmission, host investment in defense is a downward U-shaped function of vertical transmission, unlike the continuous increase seen in only-host evolution, as a result of compensatory investment by the symbiont; and (v) when hosts and symbionts are coevolving, an increase in general herbivory selects for a greater investment in defense than when either hosts are symbionts are evolving alone. While we focused on modeling a specific tripartite system, our findings are applicable to similar host–symbiont systems facing attacks from multiple herbivore/predator species, where one herbivore/predator remains indifferent to the defense while another is repelled by it. For instance, the Hawaiian Bobtail Squid (*Euprymna scolopes*) forms a symbiotic relationship with the bacterium *Vibrio fischeri* (McFall-Ngai, 2008). The host provides the bacteria with sugar and amino acid solution and in return receives bioluminescence for camouflage, which protects the squid from potential predators on the sea floor. This defense mechanism deters visual predators but not predators relying on other senses for hunting.

Theoretical investigations into the evolution of host or symbiont-mediated defense against multiple enemies have either considered multiple parasite species (Ashby & King, 2017; Rafaluk-Mohr et al., 2018; Smith & Ashby, 2023; Toor & Best, 2016), or a combination of parasite and predator (Best, 2018; Hoyle et al., 2012; Toor & Best, 2015). However, in natural systems, several predators coexist and potentially complicate the ecological dynamics (Betts et al., 2016; Zhang et al., 2014). When investment in defense compromises host reproduction, intermediate predation rates drive the selection for maximal defense against parasitism in only-host evolution scenarios (Toor & Best, 2015), and when host and parasite coevolve (Best, 2018). Our model reveals that slug herbivory affects defense evolution depending upon the associated costs: minimal defense emerges at high slug herbivory rates when hosts are under selection and costs are
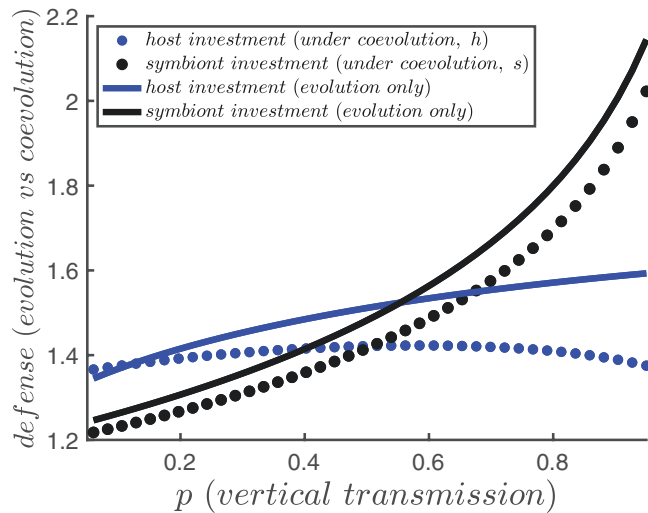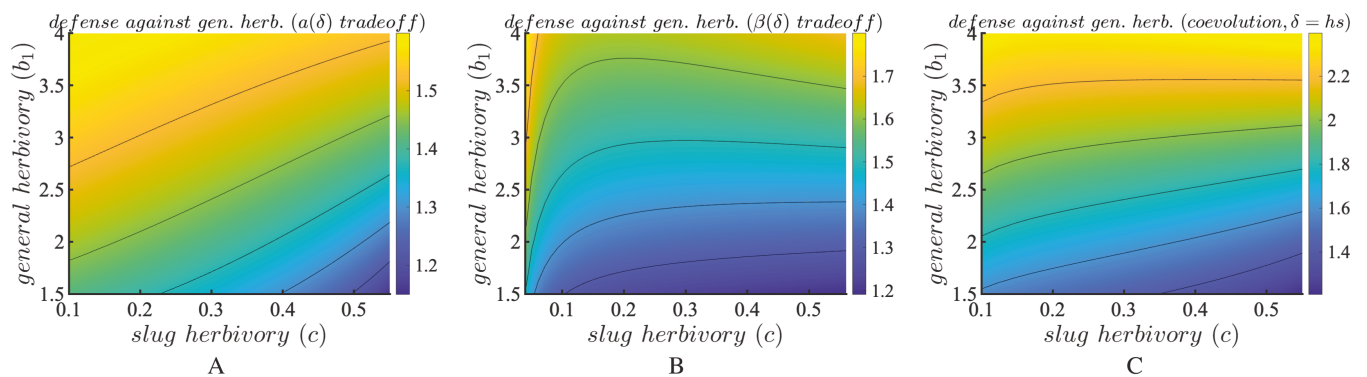
Figure 7. Contour plots showing the variation in defense against general herbivory when the cost is to (A) algal host reproduction, (B) horizontal transmission, and (C) both reproduction and transmission i.e., coevolution.

to host reproduction, and at intermediate herbivory rates when selection is on the symbiont and costs are to symbiont transmission. We also found that the coexistence of different symbiont strains can emerge through evolutionary branching, a phenomenon also observed in host–parasite–herbivore models: in parasites in the context of a transmission-virulence trade-off (Best, 2018; Morozov & Best, 2012), or in hosts as a result of a defense allocation trade-off against two enemies (Toor & Best, 2016). A relevant experiment considered the evolution of the host bacterium *Pseudomonas fluorescens* against two enemies (a virus and a predatory protist) (Friman & Buckling, 2013): the host population diversified into two genotypes, one with high defense against the virus and another with high defense against the predator, as a result of a trade-off between defenses against both enemies. Similarly, our research provides insights into possible genetic variation in the bacterium-carrying algal populations in the context of particular trade-off structures, setting the stage for future empirical investigations.

Zan et al. (2019) discovered that the bacterium residing within algal cells produces the toxic lipopeptide compound, kahalalide F. This compound plays a crucial role in this Hawaiian system by protecting *Bryopsis* sp. algae from herbivorous fish. Interestingly, the specialist herbivorous sea slug *E. rufescens* not only consumes the algae but also sequesters kahalalide F for its own defense against predators (Becerro et al., 2001; Mascuch & Kubanek, 2019). Thus, kahalalide F moves from bacteria to algae to slug, protecting multiple species and contributing to a complex marine ecosystem. This cytotoxic chemical has gained attention for its potential medicinal properties, with research identifying it as a promising treatment for conditions such as hepatocellular carcinoma, melanoma, nonsmall cell lung cancer, and psoriasis (Gao & Hamann, 2011; Wyer et al., 2022). The evolution of such natural compounds remains significantly understudied, despite decades of research into the genetic and biophysical mechanisms of bacterial natural product biosynthesis (Chevrette et al., 2020). Our model aims to fill this gap by providing insights into the evolutionary dynamics of defense (production of kahalalide F), predicting ecological conditions under which its production would increase or decrease based on the associated trade-offs. Our conclusions, however, await empirical grounding of both the underlying tradeoffs and the range of diversity observed in natural systems.

Our model opens up intriguing avenues for future research. For instance, we considered the evolution of the alga and symbiotic bacterium as separate entities. Taking a "holobiont" perspective where hosts and symbionts respond to selection as a single integrated unit (Roughgarden, 2023; Roughgarden et al., 2018) might lead to different outcomes. Further, in our model of two herbivores, we model the population dynamics of only slugs, while accounting for the impact of general herbivores simply through added mortality rates. Our conclusions about the relative magnitude of selection effects from each herbivore type may change in models that include feedbacks in the population of the second herbivore, and trade-offs between defenses against different herbivores could introduce additional complexities. Since the ratio of slugs to general herbivory remains unknown, a possible model extension could involve replacing slugs with general herbivores or focusing on the impacts of a generalist herbivore (e.g., fish) versus a specialist herbivore (slugs). Finally, determining the costs and benefits of symbiont carriage would be a crucial direction for future empirical research.

To conclude, our adaptive dynamics-based modeling framework broadly captures the selection pressures arising in this tripartite symbiosis but inevitably simplifies some aspects of

evolutionary ecology, including potential (co)-evolution by the slug, or the existence of other costs in defense beyond algal reproduction and symbiont transmission. Furthermore, the nuance of the trade-offs modeled here still lacks a robust empirical foundation. Nonetheless, our model identifies core aspects of the life history that require characterization for application to specific empirical systems and lays the foundations for extension of the theory on symbiont evolution in multi-species trophic cascades.

Supplementary material

Supplementary material is available online at *Evolution Letters*.

Data availability

MATLAB codes for the simulations are available on Figshare: https://figshare.com/articles/dataset/Characterizing_evolution_of_defense_in_a_tripartite_marine_symbiosis_system_using_adaptive_dynamics/26058133.

Author contributions

C.J.E.M. and M.S.D. conceived the study; P.S., C.J.E.M., G.D.C., and M.B. developed the methods and wrote the first draft; all authors edited and commented on the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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