# The Evolutionary Ecology of Physiological Constraints in Ecological Communities and HPV-Induced Cancer

BY

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THESIS

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This thesis is dedicated to my beautiful wife Leah.

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## SUMMARY

I use consumer resource theory and evolutionary game theory in developing theory with regards to physiology, protists, and viruses. I use differential equation consumer resource systems to provide an ecological basis for theoretical exploration. And I use game theory to model natural selection within an ecological context.

I investigate the effects of stoichiometric constraints on consumers in a graphical framework. I found that when stoichiometry is integrated into consumer resource models, ecological communities take on a more complex array of possible states. When resource equilibrium abundances are low, ecological communities are nearly identical to those used to characterize animal communities based on substitutable resources. However, when resource equilibrium abundances are high, ecological communities are similar to those used to characterize plant communities based on essential resources.

I investigate the effects of species that can switch trophic levels by a morphologic transformation on ecological communities. I found that these species often stabilize population dynamics, which favors each morph as a separate species. I conclude that switching species likely evolve in environments with stochastic resource fluctuations or extrinsic drivers of resource levels. I also found that although switching species can fill diverse ecological niches in a community, they do not necessarily restrict diversity.

I investigate the role of digestive physiology coupled with the digestive properties of resources in structuring ecological communities. I found that bulky resources select for large guts with long throughput times, and high energy/volume resources select for small guts and short throughput times. Most resource pairs lead to the evolution of a specialist on the richer resource followed by the invasion and evolution of a generalist.

I also applied theory to HPV induced cancers. I hypothesized that HPV faces a life history tradeoff, where HR HPV is persistent but not very infectious, and vice versa for low-risk HPV. We found that different sexual subcultures within the human population could explain the origin and maintenance of these distinct HPV types.

Furthermore, I made a PDE model of HPV infection within mucosal tissue to discover the links between cell population dynamics and HPV protein expression. And how somatic evolution of cells produces tissue level changes. I found that HPV's proteins likely increase the density of tissue at which cells can divide and possibly also slow the migration rate of cells to the skin surface. I also found that somatic evolution is an alternative explanation for tissue level changes observed during high-risk HPV infection.

# 1. Introduction

The goal of ecology is to understand the processes giving rise to the abundance and distribution of organisms. There are a number of different approaches to doing ecology. I take a theory-based approach using mathematical models. Following Hutchinson (1965), I use consumer resource models to represent the ecological theatre and evolutionary game theory to describe the evolutionary play. In what follows I provide background and justification to the modeling approach I use throughout this thesis.

Mathematical models are simply sophisticated hypotheses. The beauty of mathematical models is that they are correct given that their assumptions are correct. Once a general model is formulated, scientists can use data to decide which assumptions of a model are correct and which are not. I chose to work with mathematical models for my Ph.D. because I believe that scientific inquiry begins with a model describing how a given system works. Mathematical models in particular allow us to understand simple relationships between variables, which can account for complex phenomena. Furthermore, mathematical models can be naturally combined into statistical analyses. Thus, mathematical models are the appropriate tools to use in the search for general processes in ecology. Mathematical models have been successful in meeting this goal. This dissertation builds off the success of others and continues the tradition of the use of mathematical theory in ecology.

I chose consumer resource models both as a mechanistic representation of ecology and as the fundamental unit of ecological interaction. A consumer is a living organism, which consumes resources in order to reproduce. A resource can either be living or nonliving. Increases in the rate of resource consumption increase consumer

fitness (i.e. resources are limiting) and consumption depletes resources. All living organisms are consumers and the majority are both consumers and resources (i.e. organisms are links in food chains). All other ecological interactions can be understood as modifying a consumer resource interaction. For instance, in this context, mutualism is defined as the positive effect of two species on each other by reciprocally increasing access to limiting resources.

Consumer resource interactions naturally lead to a "struggle for existence", which Darwin (1859) used to describe the fact that exponential increases in population size eventually feed back through resource or predator abundances resulting in zero net per capita population growth. The struggle for existence is a centerpiece of my thesis as it is a common thread in theories of community structure and the theory of natural selection. The struggle for existence manifests as negative density dependence. Some have argued that along with exponential growth, negative density dependence is a fundamental law of population ecology (e.g. Turchin 2001). The most important ecological consequences of negative density dependence are equilibrium points. Equilibrium points may be repellors or attractors. Repellors result in ephemeral ecological associations. Attractors allow for coexistence and display a variety of behaviors from exponential stability to limit cycles to chaos. Simple consumer resource models predict all of these behaviors of communities. Moreover, all of these behaviors are seen in nature. Turchin's (2003) book on complex population dynamics provides good examples of connections between data and simple consumer resource models.

Following the theme of the struggle for existence, Tilman's (1982) theory of competition and community structure uses consumer resource models to understand

communities. An ecological community describes a group of functionally similar organisms, which share a habitat. Most of the work in this thesis builds off of Tilman's theory. Two aspects of Tilman's theory appeal to me. First, it is built upon explicit consumer resource interactions, which are fundamental ecological interactions. Second, it is currently the most successful theory describing community organization. I stress this second point, as many ecologists are discontent with Tilman's theory (e.g. Grace 1991, Craine 2005).

Tilman's theory identifies the mechanisms underlying coexistence in competition based community theory. Chesson (2000) further generalizes Tilman's conclusions to include situations where resources vary in space and time. In the end, coexistence comes down to 2 questions 1.) Is there an equilibrium point, which includes positive densities of the species in question, and 2.) Is this equilibrium point stable? In line with these questions, Chesson identifies 2 forces, which are integral to community structure - fitness equalizing forces and stabilizing forces. Fitness equalizing forces create equilibrium points and stabilizing forces stabilize them. Tradeoffs are a central concept in competition based community structure. Evolutionary tradeoffs tend to be both equalizing and stabilizing. Tradeoffs ensure that a single species is not competitively superior in all circumstances, which creates equilibrium points. Tradeoffs also generally ensure that each consumer feeds more heavily on the resource, which they are more specialized for, which reduces interspecific competition and stabilizes equilibrium points. As an aside, both predators and resources create a struggle for existence. Competition theory can also be extended to prey that share predators. Holt (1977) refers to this as apparent competition. Increasing trophic levels increases the complexity of models.

However, the 2 questions relating to biodiversity and community structure remain the same.

The alternative theory of community structure that currently receives the most attention is Hubbel's (2001) "neutral theory". The neutral theory I believe has traction with ecologists since it is a simple model, which can explain a lot of (actually infinite) biodiversity. The neutral theory is actually also based on competitive interactions between species. The critical difference between Tilman's theory and the neutral theory is that the neutral theory assumes no evolutionary tradeoffs, i.e. all species are functionally/ecologically identical. In the vein of Chesson, the neutral theory includes only equalizing forces in its simple assumption of ecological equivalence. Given this assumption, there are no distinct equilibrium points. Communities are composed of infinitely many neutral equilibrium points. Therefore, the neutral theory is really just a very restricted case of competition based community structure. In addition, most ecologists are hard pressed to believe that a large community of species are functionally equivalent.

There is ample proof that Tilman's theory works. Recently, Miller et al. 2005 reviewed all studies, which tested Tilman's theory of community structure. They found that 75% of the studies supported Tilman's theory. Admittedly, Tilman's theory is unwieldy for high diversity communities. However, that does not mean that the fundamental mechanisms of competition and coexistence that operate in simple communities do not operate in more speciose communities. Certainly, Tilman's theory of community structure is the best that we currently have.

Evolutionary ecologists see tradeoffs combined with environmental heterogeneity as the answer to the question of biodiversity. But, why do tradeoffs exist in the first place? Levins (1968) argued that given diverse environmental opportunities, evolution by natural selection pushes species to the brink of performance, where a tradeoff is naturally enforced. Thus, tradeoffs are not required. However, evolutionary biologists expect them to occur when there are distinct environmental opportunities and traits to exploit these opportunities are under selection. In other words, evolutionary biologists expect that phenotypes without tradeoffs are evolutionarily ephemeral (Tilman 1982). Tradeoffs showcase the fact that ecological communities are best understood in an evolutionary framework.

Darwin recognized that the struggle for existence combined with heritable variation creates an evolutionary dynamic. Also, evolving systems can have equilibria. There are two main consequences of evolution on ecological communities: 1. Evolutionary dynamics can lead to changes in the stability of ecological equilibria (Abrams and Matsuda 1997, Fussman et al. 2007). For example, an ecological equilibrium could change limit cycles to exponential stability under the influence of evolutionary change. Pimentel et al. (1963) demonstrated that evolution in experimental host-parisitoid systems resulted in stabilization of ecological dynamics. 2. The existence of evolutionary equilibria in communities means that there is a coalition of species that natural selection maintains, each with an evolutionarily stable niche. This second point gives us a deeper understanding of ecological communities and the niche concept. Ripa et al. (2009) refer to these evolutionary stable niches as “niche archetypes”. The communities of anoles on the Caribbean islands exemplify the idea of niche archetypes.

Losos (2009) has shown that the anoles routinely fill 5 distinct niches corresponding to vegetational microhabitats. I use routinely, in the sense that each island represents convergent evolution of the anoles into these archetypal niches.

Ecology makes more sense in an evolutionary framework. Evolution certainly makes no sense without ecology. However, during most of the last century, the development of the fields of ecology and evolution, at least theoretically, were largely separate. The application of game theory to evolution helped to bridge the gap between ecology and evolution. An evolutionary game describes a situation where the fitness of individuals using a particular strategy (i.e. phenotype) depends on the strategies used by other individuals in the population. Evolutionary game theory formalized the idea of evolutionary equilibria with the evolutionarily stable strategy (ESS). An ESS is uninvadable by any potential rare mutants in the population (Maynard-Smith 1982). It was not until the 80's that Brown and Vincent united theoretical ecology and evolution by building continuous strategy games into the differential equation models of classic theoretical ecology.

I use Vincent and Brown's (2005) G-function approach to model evolution in chapters 4 and 5. A particular G-function is an equation for the per capita growth rate of individuals (i.e. fitness) as a function of ecological circumstances and evolutionary strategy. G-functions have the virtue of setting evolution in an explicitly ecological context. This allows for the complete generalization of natural selection. The ecological dynamics generalize the effects of density on fitness. Including density dependent (positive or negative) or independent selection. This combined with the generalization of frequency dependent effects by game theory allows for the description of any type of

natural selection. Genetical models of evolution either assume infinite population size (destroying most of the interesting ecology) or study evolution in an individual based framework, which precludes the simplicity of mathematical analysis in understanding the emerging patterns.

G-functions allow us to understand both ecology and evolution as part of the same dynamical system. Admittedly, much of the theory is hard to test and remains untested. For example, the debate over whether evolutionarily stable minima are long-term stable or even common in nature remains entirely unresolved (Abrams 1993, Reuffler et al. 2006). Notably, however, work from Michael Doebeli's lab has demonstrated adaptive speciation in bacteria (Friesen et al. 2004).

In summary, the struggle for existence provides a unifying theme for the study of ecology and evolution. Consumer resource interactions push species towards ecological equilibria, where a struggle for existence ensues. This simple notion has far reaching consequences for the abundance and distribution of organisms. I apply these fundamentals of ecology and evolution to a variety of situations from physiological constraints on consumers to HPV induced cancer.

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## 2. Coexistence and Community Structure in a Consumer Resource Model with Implicit Stoichiometry

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### 2.1 Introduction

Consumer resource theory historically focused on energetic rewards of substitutable resources. Stoichiometry theory (Sterner and Elser, 2002) allows for multiple chemical substances to limit consumers. Stoichiometric constraints on the consumer may drive complementary or essential relationships between resources. Thus, integration of stoichiometry into the consumer-resource framework should enhance theoretical understanding of community function and structure. Four observations support this assertion: 1) Consumers require a balanced diet for optimal growth and reproduction (e.g. Behmer 2009). 2) Chemical composition varies widely across potential resource species (e.g. Frost et al., 2003; Evans-White et al., 2005). 3) Generalist heterotrophs are common in nature (e.g. Polis 1991). 4) Consumers exhibit adaptive behaviors that regulate nutrient intakes (e.g. Mayntz et al., 2005).

Limitation by multiple chemical elements may be a general phenomenon across trophic levels (e.g. Tilman, 1982; Mayntz et al., 2005; Behmer 2009). Central to modeling the consequences of this limitation is the concept of "threshold elemental ratios" (TER; Urabe and Watanabe 1992), which stoichiometrically embodies the idea of nutrient balancing. A given $TER_{X:Y}$ represents the dietary ratio of element X to element Y at which the consumer is equally limited by both elements. Consumers feeding on a

diet with a higher ratio will be limited by element Y, and consumers feeding on a diet with a lower ratio will be limited by element X. Empirical studies support these theoretical predictions of TER (Sterner and Elser 2002). Most TER studies have focused on zooplankton, although the same pattern of nutrient limitation arises in terrestrial arthropods fed different ratios of carbohydrate:protein (e.g. Simpson et al., 2002; Mayntz et al., 2005; Raubenheimer and Jones 2006). Carbohydrate:protein ratios are closely associated with C:N ratios. Available resources often vary in chemical composition (Sterner and Elser 2002). For example, C:N ratios of autotroph tissues can vary considerably within an individual, whereas insect tissues exhibit little intra-individual variation (tight homeostatic control), but C:N ratio can vary 3-fold across insect taxa. Elemental composition of insects varies with both phylogeny and trophic position (Fagan et al., 2002; Woods et al., 2004).

Given this variation in the elemental composition of resources, and the uncertainty that can accompany reliance on a specific resource type, it is often, but not necessarily always the case, that the best strategy for a consumer may be a mixed diet. The selective pressure that nutrient balancing places upon a consumer has been proposed as a mechanism driving the evolution of generalist consumers (Bernays and Minkenberg 1997). There is certainly a preponderance of generalists within natural communities (e.g. Polis 1991), irrespective of the selective pressures and other causes that could lead to generalist diets.

The evolution of a generalist diet leads to the question of which diet-selection behaviors are adaptive under particular conditions. Abrams (1987) modeled the functional response of adaptive foragers utilizing two resources each containing essential

nutrients. He found that a consumer's adaptive foraging behavior resulted in functional responses that were highly divergent from the shape of the underlying type II functional response. This finding is important because the form of a consumer's functional response in one factor affecting the behavior of consumer-resource systems (Abrams 1980). Empirical studies of diet selection confirm that foraging consumers can balance their diets in an adaptive manner. Simpson et al. (2004) found that the generalist caterpillar, *Spodoptera littoralis*, selects a diet that coincides with the peak of an experimentally determined fitness landscape, where the independent variables of the landscape were carbohydrate and protein intakes. Such adaptive behaviors indicate that stoichiometry may be an important factor in the evolution of heterotroph communities.

In our model we incorporate resource stoichiometry and adaptive feeding behaviors into a consumer-resource population model in order to investigate consequences of stoichiometry on heterotroph communities. Most research on the consequences of introducing stoichiometry into consumer-resource models has focused on phytoplankton and/or zooplankton systems – modeling passive/specialist foragers (for reviews see Andersen et al., 2004; Hall 2009). We add to this existing body of theory by including adaptive foraging by generalist consumers. An advantage of our approach is that by focusing on a generalist forager, we can derive predictions of tradeoffs and competitive coexistence (e.g. Vincent et al., 1996), subjects generally absent from existing stoichiometry theory (but see Hall 2004; Loladze et al., 2004). In general, our model differs from previous stoichiometric consumer-resource theory, as we do not assume dynamic feedbacks between the consumer, resource elemental composition, and inorganic nutrient supplies (but see Fryxell and Lundberg 1998). Therefore, we refer to

our representation of stoichiometry as implicit. Our model most readily applies to higher trophic levels (i.e. secondary consumers and above), as we assume that resource elemental compositions remain fixed.

In developing our model we incorporate elements of Tilman's (1980, 1982) graphical theory to produce consumer Zero Net Growth Isoclines (ZNGIs), and we borrow from Rosenzweig (1981) to produce Isolegs, which describe the adaptive behavior of the consumer. We utilize consumer consumption vectors to investigate tradeoffs in resource and elemental use efficiency leading to coexistence.

### 2.2 Model Description

We modify MacArthur's (1972) consumer resource model to include consumers with stoichiometric constraints. The model consists of two co-occurring resources consumed by two generalist consumers. We assume that the resources contain different ratios of the elements X and Y, and that the consumption and conversion of these elements determines consumer fitness.

Let the growth rate of consumer j (j =1,2) be given by equation 1, where $N_j$ is the density of individuals of consumer j, $e_{Xj}$ and $e_{Yj}$ are the conversion efficiencies (individuals of consumer j/unit of element consumed) of elements X and Y into new consumers, and $C_{Xj}$ and $C_{Yj}$, the per capita consumption rates (amount of each element consumed x time$^{-1}$ x consumer j$^{-1}$) of elements X and Y, respectively:

$$\frac{dN_j}{dt} = N_j\left[\min\left(e_{Xj}C_{Xj}, e_{Yj}C_{Yj}\right) - m_j\right] \quad (1)$$

The minimum function determines the ratio at which the two elements simultaneously limit consumer growth – analogous to a TER. Setting the conversion rates of each

element equal gives the ratio $C_X/C_Y = e_Y/e_X$, which can be thought of as an optimal ratio of consumption of the two elements. The death rate of the consumer is given by a density-independent per capita mortality rate $m_j$ (time$^{-1}$) multiplied by the number of consumers.

We model the functional response of each consumer to each resource with a two-resource version of Holling's disk equation (Holling 1959):

$$C_{ij} = \frac{p_{ij} a_{ij} R_i}{1 + \sum_{i=1}^{2} p_{ij} a_{ij} R_i h_{ij}} \qquad (2)$$

Resource consumption is a function of the consumer's resource garnering traits (encounter efficiencies and handling times), the abundance of both resources ($R_i$, where i=1,2), and the consumer's adaptive diet-selection behavior; where $a_{ij}$ (time$^{-1}$) is the encounter efficiency of consumer j on resource i, and $h_{ij}$ (time/resource) is the handling time of consumer j on resource i. Note that over-consumption of a particular resource negatively impacts consumer fitness only via handling-time costs. Handling times are then what drive adaptive diet selection in the model. We integrate adaptive behavior into the model with a decision variable $p_{ij}$, which gives the probability that consumer j accepts an encountered item of resource i.

Following from the functional response formula, we model the per capita consumption rates of each element by multiplying the resource abundances in the functional response formula by the amounts of elements X (equation 3) or Y (equation 4) contained in each resource, and then summing over resources to include the total amount of each element consumed. For simplicity, we assume that each resource has weight

equal to one, but contains different fractions of each element, where here $x_i$ and $y_i$ are the fractions of element X and Y, respectively, contained in resource i:

$$C_{Xj} = \frac{\sum_{i=1}^{2} p_{ij} a_{ij} R_i x_i}{1 + \sum_{i=1}^{2} p_{ij} a_{ij} R_i h_{ij}} \tag{3}$$

$$C_{Yj} = \frac{\sum_{i=1}^{2} p_{ij} a_{ij} R_i y_i}{1 + \sum_{i=1}^{2} p_{ij} a_{ij} R_i h_{ij}} \tag{4}$$

Finally, we model resource dynamics assuming that resource populations grow logistically and die from consumer predation (equation 5). Here, $R_i$ is the density of individuals of a particular resource (i=1,2), $r_i$ is the resource intrinsic growth rate (time$^{-1}$), and $K_i$ (density of individual resources) is the resource carrying capacity in the absence of consumers. Resource consumption is described by the functional response given above (equation 2) multiplied by the number of consumers.

$$\frac{dR_i}{dt} = R_i r_i \left(1 - \frac{R_i}{K_i}\right) - \sum_{j=1}^{2} N_j C_{ij} \tag{5}$$

First we examine the consequences of different resource stoichiometries on the foraging behavior of a single generalist consumer. We then examine how resource stoichiometry can influence the tradeoffs that can affect how two competing generalist consumers can coexist. Finally, we study how different assumptions about the tradeoffs can influence communities.

## 2.3 Results

### 2.3.1 *Effects of Different Resource Stoichiometries On the Foraging Behavior and Resource Requirements of a Single Consumer*

We examine the implications of two fundamentally different cases of consumer resource stoichiometry: (1) the case where the elemental ratios of both resources are greater (or lesser) than the consumer's TER, which leads to limitation by a single element; (2) the case where the elemental ratio of one resource is higher than, and that of the other resource lower than, the consumer's TER, which gives the consumer the ability to balance the intake of elements in its diet.

We integrate adaptive behaviors, interactions between consumer and resources, and population dynamics, with isolegs (Rosenzweig 1981) and Zero Net Growth Isoclines (ZNGIs, Tilman 1982). Isolegs graphically display consumer adaptive behaviors in the state space of resource abundances. An isoleg describes all combinations of $R_1$ and $R_2$ where two different foraging strategies result in equal fitness payoffs (per capita growth rates). On either side of the isoleg, fitness payoffs of the foraging strategies differ, and optimal consumers utilize different adaptive foraging strategies. Foraging decisions influence resource relationships and population dynamics of resources and consumers within the system. ZNGIs depict these relationships graphically. A ZNGI is a curve in the resource state space that describes all combinations of $R_1$ and $R_2$ such that the consumer species' population growth rate is zero (Tilman 1982).

For resources with elemental ratios both less than the consumer's TER, we assume $e_y{:}e_x > x_1{:}y_1 > x_2{:}y_2$, where $x_1 > x_2$ and $y_2 > y_1$. In this case, no matter how a

consumer selects its diets, it will always be taking in too much Y and not enough X. Therefore, it will always be X-limited. Limitation by a single element leads to substitutable resources. If we assume equal handling time and encounter efficiency for each resource, then $R_1$ is utilized more efficiently than $R_2$. The slope of the consumer's ZNGI will be less than -1, which gives the negative of the relative values of the two resources (marginal rate of substitution, see below for further explanation). As the equilibrium level of $R_1$ increases, its value relative to $R_2$ also increases, since $R_2$ does not yield as much Y per unit time, and thus harvesting $R_2$ takes away from time that could be spent searching for, and handling, $R_1$. If $R_1$ is abundant enough, the consumer benefits from being selective ($p_1$=1, $p_2$=0) on $R_1$ and ignoring encountered items of $R_2$. Therefore the isoleg is a vertical line in resource state space denoting a specific value of $R_1$ (i.e. the zero-one rule, Pulliam 1974; Stephens and Krebs 1986).

Two papers have thoroughly reviewed the consumer-resource theory of substitutable resources (Brown and Mitchell 1989; and Vincent et al., 1996). Therefore, we will not discuss it any further here. We point out that the condition of substitutable resources results from a single limiting element, and can arise as a special case of consumer-resource stoichiometry. As we explore further the consequences of different resource stoichiometries, we will frequently contrast the case of a single limiting element to novel predictions arising from limitation by >1 element.

As our second case, we consider resources with elemental ratios that straddle the consumer's TER. We assume that $x_1{:}y_1 > e_y{:}e_x > x_2{:}y_2$. Depending upon resource abundances and how the consumer selects among them, the consumer's population can be limited by either X or Y. To determine which element is limiting, we define and derive

the Equal Limitation Line (ELL), which describes all combinations of $R_1$ and $R_2$ for which an opportunistically foraging consumer is simultaneously limited by both X and Y. The slope of the ELL gives the ratio of resource consumption for which the two elements equally limit the consumer. Setting the per capita birth rates of the consumer from elements X and Y equal, and solving for $R_2$, yields the equation for the ELL in resource state space (Equation 6). The ELL is depicted graphically in Fig.1 as the dotted line crossing the origin and bisecting the resource state space. Above the ELL the consumer is limited by X, and below the ELL, by Y:

$$R_2 = \frac{a_1 R_1 \left( e_X x_1 - e_Y y_1 \right)}{a_2 \left( e_Y y_2 - e_X x_2 \right)} \tag{6}$$

Once the forager recognizes an elemental deficiency, it initiates a foraging strategy that maximizes the intake of this element. The appropriate foraging strategy depends upon the abundance of the resource containing relatively more of the limiting element. For example, if a consumer is limited by X, this consumer is more limited by food 1 because it contains a higher X:Y ratio than food 2. When food 1 is rare in the environment, the consumer may consume more X per unit time by foraging opportunistically. On the contrary, when food 1 is abundant, the consumer should forage partially selectively on food 1 ($p_1$=1, $p_2$<1). Partial-selectivity occurs when a consumer always accepts one food item when encountered (is partially selective, but not fully selective on this food item), but accepts only some of the other food item when encountered. This behavior results because, under X-limitation, the consumer should always consume food 1 when encountered, but, to ensure that Y does not become limiting, the consumer also needs to consume sufficient amounts of food 2. Thus, the isoleg that separates regions of opportunism from partial selectivity on food 1 represents

a specific abundance of food 1. Setting the harvest rates of X from a generalized diet ($p_1$=1, $p_2$=1) and a specialized diet ($p_1$=1, $p_2$=0) equal and solving for $R_1$ yields this isoleg (equation 7). The same logic can be used to solve for the Y-limited isoleg (equation 8). These isolegs produce vertical and horizontal lines for X and Y limitation, respectively.

$$R_1 = \frac{x_2}{a_1\left(h_2 x_1 - h_1 x_2\right)} \tag{7}$$

$$R_2 = \frac{y_1}{a_2\left(h_1 y_2 - h_2 y_1\right)} \tag{8}$$

The two isolegs combined with the ELL divide the state space into three areas of distinct foraging strategies – opportunistic on both foods 1 and 2, partial selectivity on food 1, and partial selectivity on food 2 (Fig. 2.1). In the regions where a consumer forages partially selectively, it accepts all of one of the resources and only enough of the other to maintain equal limitation by both elements. In solving for the adaptive partial selectivity, the per capita birth rates from elements X and Y are set equal, $p_i$ of the limiting resource is set equal to one, and the equation is solved in terms of $p_i$ of the other, non-limiting resource. The optimal partial selectivity depends upon relative resource encounter rates and the mismatch between the consumer's stoichiometric demands and resource elemental ratios (equations 9 and 10). When partial selectivity is optimal, then

$$p_1 = \frac{a_2 R_2\left(e_Y y_2 - e_X x_2\right)}{a_1 R_1\left(e_X x_1 - e_Y y_1\right)}, \text{ when } p_2=1 \tag{9}$$

and

$$p_2 = \frac{a_1 R_1\left(e_X x_1 - e_Y y_1\right)}{a_2 R_2\left(e_Y y_2 - e_X x_2\right)}, \text{ when } p_1=1. \tag{10}$$

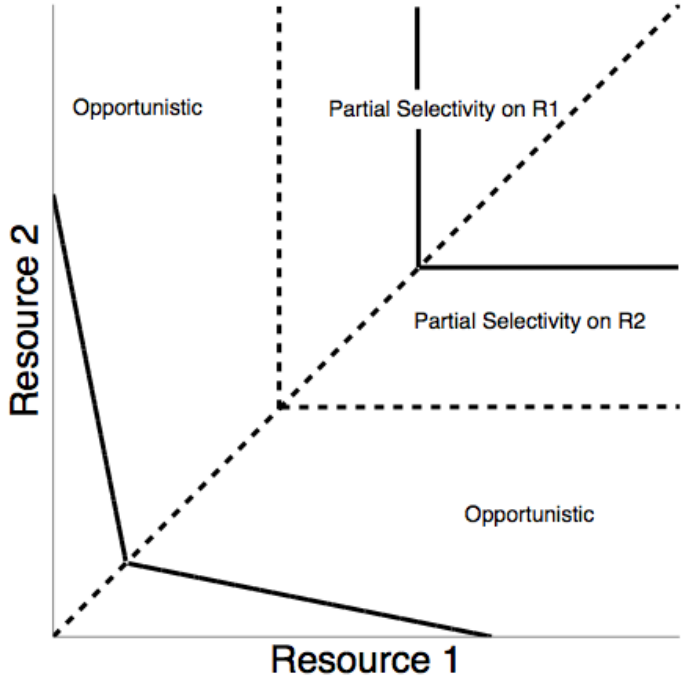


**Figure 2.1:** Behavioral isolegs and Zero Net Growth Isoclines of a generalist consumer with stoichiometric constraints. Behavioral isolegs (dashed lines) separate regions of different adaptive foraging behaviors, which are labeled in the figure. The dashed line with positive slope is the consumer's Equal Limitation Line, along which both nutrients are equally limiting. Below the ELL the consumer is limited by nutrient Y and above by nutrient X. Vertical and horizontal dashed lines separate regions of opportunism from regions of partial selectivity on Resource 1 and 2, respectively. The solid lines represent the consumers ZNGI under low and high resource equilibrium abundances. Low resource equilibrium abundances promote opportunistic foraging, and complementary resources as depicted by the ZNGI closer to the origin. High resource equilibrium abundances promote partially selective foraging, and essential resources as depicted by the ZNGI farther from the origin.

ZNGI's aid in visualizing the population dynamics of consumers in the state space of resource abundances. A consumer's ZNGI represents its subsistence level of resources. We can solve for the ZNGI by substituting adaptive foraging strategies into the consumer's fitness function, setting the fitness function equal to zero, and then

solving for $R_2$. The ZNGI needs to be solved separately for both X and Y limitation – the actual ZNGI is then the outer boundary of the two. This generates ZNGI's that have discontinuities where the two "pieces" intersect.

When consumers require relatively low subsistence levels of resources, consumers adaptively forage by selecting their diets opportunistically. This leads to a complementary ZNGI, which is depicted in Fig. 1 as the two solid lines closer to the origin. Resources are substitutable to one another under either X or Y limitation (equation 11), but their relative values change depending upon which element is limiting.

$$R_2 = \begin{cases} \dfrac{R_1 a_1 \left(m h_1 - e_X x_1\right) + m}{a_2 \left(e_X x_2 - m h_2\right)} & \text{if x-limited} \\ \dfrac{R_1 a_1 \left(m h_1 - e_Y y_1\right) + m}{a_2 \left(e_Y y_2 - m h_2\right)} & \text{if y-limited} \end{cases} \tag{11}$$

When a consumer has a relatively high subsistence level of resources, it adaptively forages by having a partially selective diet. This result leads to an essential ZNGI (equation 12), which is depicted in Fig. 2.1 as the two solid lines farther from the origin.

$$\begin{cases} R_1 = \dfrac{m\left(e_Y y_2 - e_X x_2\right)}{a_1\left[\left(e_Y y_2 - e_X x_2\right)\left(e_X x_1 - m h_1\right) + \left(e_X x_1 - e_Y y_1\right)\left(e_X x_2 - m h_2\right)\right]} & \text{if x-limited} \\ R_2 = \dfrac{m\left(e_X x_1 - e_Y y_1\right)}{a_2\left[\left(e_Y y_2 - e_X x_2\right)\left(e_Y y_2 - m h_1\right) + \left(e_X x_1 - e_Y y_1\right)\left(e_Y y_1 - m h_2\right)\right]} & \text{if y-limited} \end{cases} \tag{12}$$

### **2.3.2** *Stoichiometry, Tradeoffs, and the Coexistence of Two Competing Consumers*

#### **2.3.2.1** *Conditions for Coexistence*

The coexistence of two competing consumer species requires that the ZNGI's of the two consumers intersect, i.e. each species must have resource abundances where it is above its subsistence level of resources and its competitor is below its subsistence level. Furthermore, stable competitive coexistence in this model also requires that each consumer deplete relatively more of the resource that limits it the most relative to the other consumer (Tilman 1982; Chesson 2000). Intuitively, this condition ensures that interspecific competition is decreased more relative to intraspecific competition. Tradeoffs in each consumer's ability to find, handle, and assimilate resources can lead to stable competitive coexistence (Vincent et al., 1996).

In our model we can investigate opportunities for coexistence by permitting tradeoffs between elemental conversion efficiency – an element-garnering trait – and two resource-garnering traits – encounter efficiency and handling time. Through the integration of consumer adaptive behaviors into consumer functional responses, all of these traits have the potential to affect how much of each resource is consumed. In this section we investigate which of these potential tradeoffs can promote consumer coexistence and under what conditions these tradeoffs will allow coexistence.

Consumption vectors graphically display (Figs. 2.2-4) the consumer's rate of consumption of each resource. The slope of a consumption vector is the amount of $R_2$ consumed per unit of $R_1$ consumed. Adaptive consumers use one of two potential foraging strategies – opportunistic or partially selective. In order to derive the slopes of the consumption vectors for each of these foraging strategies, the appropriate expression

for the adaptive foraging variable is substituted into the consumer's functional response, and the per capita consumption rate of $R_2$ is divided by the per capita consumption rate of $R_1$. Equations 13 and 14 give the expressions for the slopes of the consumption vectors for opportunistically foraging and partially selectively foraging consumers, respectively.

$$\frac{R_2 a_2}{R_1 a_1} \tag{13}$$

$$\frac{e_X x_1 - e_Y y_1}{e_Y y_2 - e_X x_2} \tag{14}$$

To determine which resource is relatively more limiting to each consumer, we use the "Marginal Rate of Substitution" (MRS) of resources. The MRS describes the amount of resource 2 a consumer would be willing to exchange for one unit of resource 1. Equation 15 gives a mathematical description of the MRS. By comparing consumers' MRS's we can determine which resource is relatively more limiting to each consumer. For instance, if consumer 1's MRS is greater than that of consumer 2, we conclude that consumer 1 is relatively more limited by resource 1 and consumer 2 is relatively more limited by resource 2. The magnitude of the slope of a ZNGI happens to be the MRS. Therefore, ZNGIs can be used to graphically determine the necessary differences in consumption vectors. These differences are a necessary but not sufficient condition for stable coexistence of consumers.

$$MRS_{12} = \frac{\partial\left(\frac{dN_i}{N_i dt}\right) \Big/ \partial R_1}{\partial\left(\frac{dN_i}{N_i dt}\right) \Big/ \partial R_2} \tag{15}$$

Finally, for resources to be in equilibrium, consumption vectors describing rates of resource depletion must have an equal and opposite counterpart – resource supply

vectors, which describe rates of resource renewal. We use the slope of the resource supply vector in conjunction with the slopes of consumers' consumption vectors to determine resource equilibrium. The slope of the resource supply vector describes the amount of $R_2$ supplied per unit of $R_1$ supplied (equation 16). Graphically, the resource supply vector must be contained within the area delimited by the slopes of the consumers' consumption vectors for the resources to be in equilibrium (Figs. 2.2-4). Resource supply vectors are a function of resource supply points ($K_1$,$K_2$), so different resource supply points will lead to different community outcomes. In general, stronger tradeoffs in the consumers' consumption vectors lead to a wider range of resource supply ratios over which resources are at equilibrium at a given consumer equilibrium point, i.e. where ZNGIs cross.

$$\frac{r_2 R_2\left(1-\frac{R_2}{K_2}\right)}{r_1 R_1\left(1-\frac{R_1}{K_1}\right)} \tag{16}$$

We consider three different consumer tradeoffs that may promote coexistence. From the consumers' consumption vector slopes (equations 13 and 14), we see that only encounter efficiencies ($a_{ij}$) and elemental conversion efficiencies ($e_{ij}$) can affect these slopes and thus allow coexistence. Tradeoffs in resource handling times cannot be a mechanism of coexistence in our model. Consequently, the three cases we consider are tradeoffs in encounter efficiencies, tradeoffs in elemental conversion efficiencies, and tradeoffs in both encounter efficiencies and elemental conversion efficiencies.

**2.3.2.2** *Tradeoffs in $a_{ij}$'s*

Only tradeoffs in encounter efficiencies ($a_{ij}$) can promote coexistence among opportunistically foraging consumers. Elemental conversion efficiencies are absent in consumption vectors of opportunistic foragers (Equation 13). Plotting the ZNGI's of two consumers with tradeoffs in encounter rates reveals three consumer equilibrium points (Fig. 2.2). In Fig. 2.2 consumer 1 (dashed lines) encounters resource 1 more efficiently and consumer 2 (solid lines) encounters resource 2 more efficiently. Therefore, the slope of the consumption vector of consumer 1 is always less than that of consumer 2. Given these tradeoffs in encounter efficiencies, the two outside equilibrium points meet the tradeoff stability condition, and the inside equilibrium point is unstable.

Based on the consumers' ZNGI's and consumption vectors, we can divide the state space into eight regions (denoted by Roman numerals; Fig. 2.2). With resource-supply points in area I, consumers will go extinct because maximum resource availabilities are below subsistence. For resource-supply points that are above consumer ZNGIs, community outcomes change along gradients of resource supply ratios. When resource supply vectors point into regions II and VI, consumer 2 will outcompete consumer 1; in regions IV and VIII consumer 1 will outcompete consumer 2. Stable coexistence occurs when resource supply vectors point into regions III and VII. Depending on the strength of the tradeoff in encounter efficiencies, regions III and VII may both be stable over a range of resource supply points, in which case the outside equilibrium points represent alternate stable states. Lastly, resource supply vectors in region V lead to a priority effect, since the equilibrium point does not meet the tradeoff

stability condition. Which consumer outcompetes the other depends upon the initial densities of each.

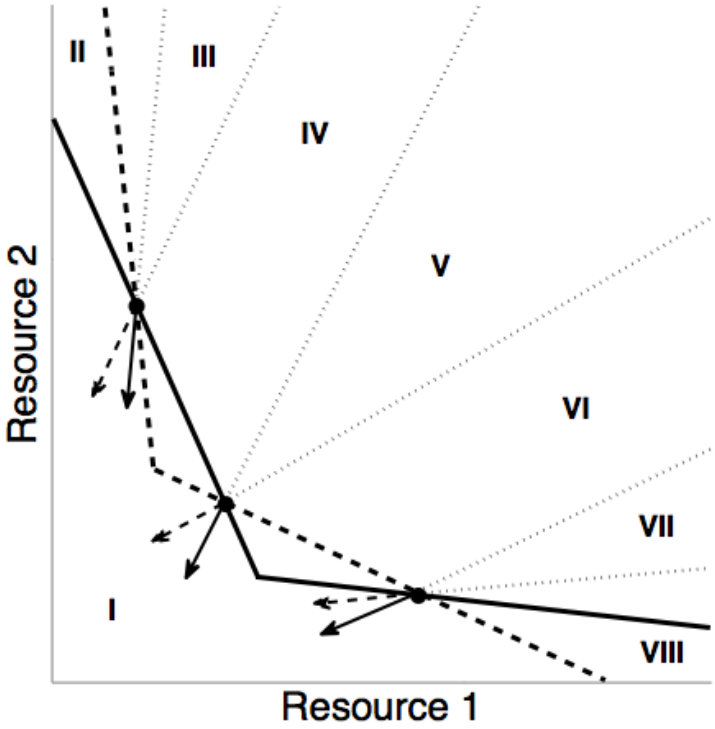


**Figure 2.2:** ZNGIs and consumption vectors of two consumers with tradeoffs in encounter efficiencies. ZNGIs (negatively sloped lines) and consumption vectors (positively sloped vectors with arrows) of consumers 1 and 2 are represented by dashed and solid lines, respectively. Roman numerals designate different community outcomes dependent upon resource dynamics. If points of resource supply occur in area I below the consumers' ZNGI's, both consumers will go extinct. For resource-supply points above consumer ZNGI's, the roman-numeral labeled areas separated by dotted lines describe different community outcomes that result when the resource-supply vector is oriented between the lines. In regions II and VI consumer 2 outcompetes consumer 1. In regions IV and VIII consumer 1 outcompetes consumer 2. In regions III and VII consumers coexist. Finally, region V leads to a priority effect among consumers.

#### **2.3.2.3** *Tradeoffs in $e_{kj}$'s*

Tradeoffs in elemental conversion efficiencies ($e_{kj}$) can promote coexistence between partially selectively foraging consumers. Resource equilibrium abundances must be relatively high to ensure coexistence. The intersection of the two species' ZNGI's for partially selective foragers shows the two-consumer equilibrium point (Fig. 2.3). In Fig. 2.3 consumer 1 is limited by resource 2 and consumer 2 is limited by resource 1. The slopes of the consumers' consumption vectors show that each consumer consumes relatively more of its own limiting resource. A tradeoff in elemental conversion efficiencies ensures that this is true through its effect on foraging behavior. However, as we show in the next section, this is not necessarily true when considering multiple tradeoffs. The equilibrium point with tradeoffs solely in elemental conversion efficiencies meets the tradeoff condition necessary for stable coexistence.

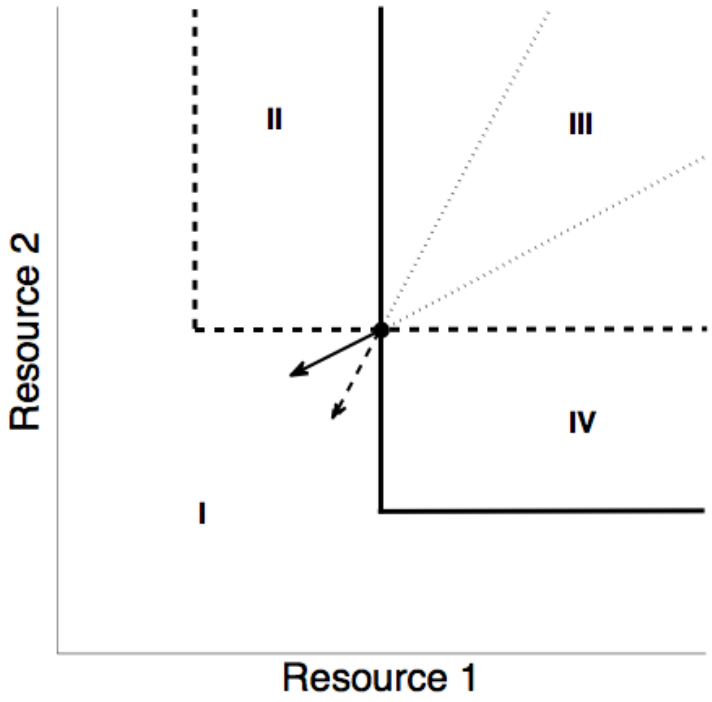


**Figure 2.3:** ZNGI's and consumption vectors of two consumers with tradeoffs in elemental conversion efficiencies. ZNGIs (negatively sloped lines) and consumption vectors (positively sloped vectors with arrows) of consumers 1 and 2 are represented by dashed and solid lines, respectively. Roman numerals designate different community outcomes dependent upon resource dynamics. If resource supply points occur in area I below the consumers ZNGIs both consumers will go extinct. For resource supply points that are above consumer ZNGIs, the areas separated by dotted lines labeled with roman numerals describe different community outcomes given that the resource supply vector is oriented between the lines. In region II consumer 1 outcompetes consumer 2. In region III both consumers coexist. Finally, in region IV consumer 2 outcompetes consumer 1.

#### **2.3.2.4** *Tradeoffs in both $a_{ij}$'s and $e_{kj}$'s*

Consumers that possess tradeoffs in both encounter efficiencies and elemental conversion efficiencies have the opportunity to coexist under both low and high equilibrium-resource abundances. There are two distinct tradeoff scenarios that we consider. The first scenario occurs when each consumer utilizes a single resource better than the other consumer, in terms of both encounter efficiency and elemental conversion

efficiency. For instance, consumer 1 may encounter resource 1 more efficiently and convert resource 1 more efficiently (via increased conversion efficiency of element X), and consumer 2 may utilize resource 2 in a similar fashion. This scenario leads to similar community outcomes as shown in Figs. 2.2 & 2.3 with, for example, low and high mortality rates, respectively.

The second scenario occurs when each consumer has a particular advantage and disadvantage on each resource type. For instance, consumer 1 may convert resource 1 more efficiently (via increased conversion efficiency of element X) and encounter resource 2 more efficiently, and vice versa for the aptitudes of consumer 2. Coexistence in this second scenario relies on relatively strong tradeoffs in elemental conversion efficiencies and relatively weak tradeoffs in encounter efficiencies. Provided these tradeoffs hold, under low resource equilibrium abundances opportunistically foraging consumers can coexist at a single equilibrium point (Fig. 2.4). Higher resource equilibrium abundances, which result in partially selective foragers, lead to similar community outcomes (Fig. 2.3).

Interestingly, considering the reverse situation – strong tradeoffs in encounter rates and relatively weak tradeoffs in elemental conversion efficiencies, the result is a single unstable equilibrium point (priority effect) with high resource equilibrium abundances. Low resource equilibrium abundances result in communities similar to those produced by tradeoffs solely in resource encounter rates (Fig. 2.2).

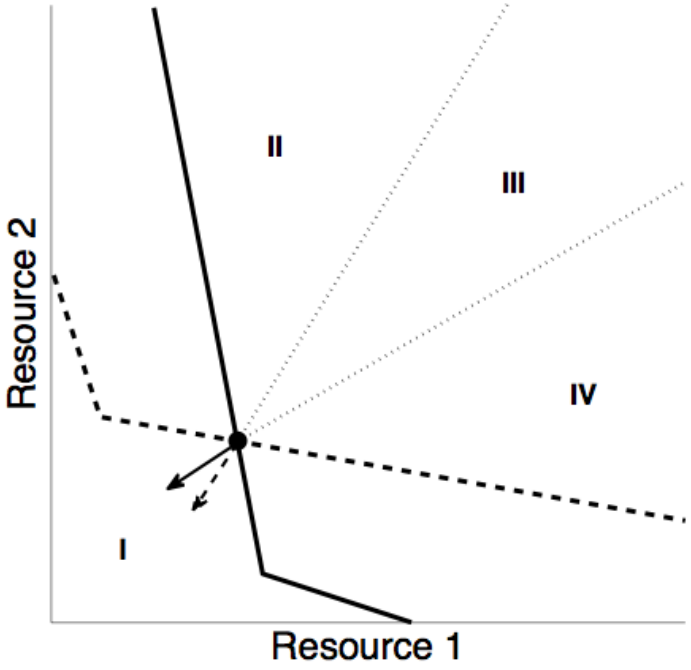


**Figure 2.4:** ZNGI's and consumption vectors of two consumers with tradeoffs in both $a_{ij}$'s and $e_{kj}$'s. ZNGIs (negatively sloped lines) and consumption vectors (positively sloped vectors with arrows) of consumers 1 and 2 are represented by dashed and solid lines, respectively. Roman numerals designate different community outcomes dependent upon resource dynamics. If resource supply points occur in area I below the consumers ZNGIs both consumers will go extinct. For resource supply points that are above consumer ZNGI's, the areas separated by dotted lines labeled with roman numerals describe different community outcomes given that the resource supply vector is oriented between the lines. In region II consumer 1 outcompetes consumer II. In region III the consumers coexist. Finally, in region IV consumer 2 outcompetes consumer 1.

## 2.4 Discussion

### 2.4.1 *Context*

Stoichiometric constraints allow for a richer set of community behaviors than strictly energetic models (for reviews see Andersen et al., 2004 and Hall 2009). The model we explored contributes to this developing theory by highlighting how foraging behavior and resource garnering tradeoffs are critical to the predictions of stoichiometry

theory at the community level. Different diet-selection behaviors combined with stoichiometric constraints produce communities based on substitutable, complementary, or essential resources, each with unique properties. Our article focuses on the more interesting cases of complementary and essential resources. These cases occur when the elemental ratios of the resources straddle the consumer's optimal elemental ratio (i.e. TER; Urabe and Watanabe 1992). Thus, the resources permit the consumer to be limited by either element. In the context of our model, these conditions are interesting because they define when our multiple currency approach produces different outcomes from a single currency approach.

### **2.4.2** *Stoichiometry and Complementary Resources*

Efficient consumers deplete resources to low equilibrium abundances. When resources are scarce, consumers forage optimally by being opportunistic and accepting all encountered resources. For instance if a consumer is limited by element X, and both resources are scarce, opportunism is the optimal foraging strategy if the consumer can harvest more X per unit time when specializing on the resource with more of element Y in comparison to specializing on the resource with more of element X. Opportunistic foraging leads to complementary resources, since each resource alone can support the consumer, but the resource with the higher X:Y is more valuable when the consumer is X-limited and vice versa for Y-limitation. These conditions lead to the same foraging behaviors and mechanism of coexistence yielded by models of substitutable resources (Vincent et al., 1996). In these models, coexistence is due to tradeoffs in resource encounter rates. However, unlike models of communities based on substitutable

resources and limitation by a single currency, the model we investigated can have three interior equilibrium points, provided that tradeoffs in resource encounter rates are stronger than tradeoffs in elemental conversion efficiencies. Thus, when consumer requirements for resources are low, communities with stoichiometric constrainsts have properties similar to communities based on single currency limitation. However, stoichiometry can lead to a more complex terrain of community stability, in which the community has an unstable equilibrium point flanked by two stable attractors, each predicting the coexistence of consumers, but with different relative abundances.

Previously developed theory of consumer coexistence in which resource stoichiometry is incorporated into the model also predicts alternate stable states (Hall 2009). Stoichiometric constraints cause kinks in consumer isoclines, which allow them to cross with the prey's isocline in multiple places, or as in the case of our model, with other consumers' isoclines. Andersen et al. (2004) review evidence for stoichiometry-driven alternate stable states in herbivore autotroph systems. Alternate stable states, in the form of multiple interior equilibrium points, appears to be a robust prediction of stoichiometry theory that occurs under a limited set of parameter values.

### **2.4.3** *Stoichiometry and Essential Resources*

Equilibrium abundances at relatively high resource levels result in communities based on essential resources. Interestingly, these communities display the same basic properties as the plant communities characterized by Tilman's (1982) original consumer resource theory. Abundant resources cause consumers to forage partially selectively, consuming just enough of each resource to balance elemental intake to an optimal ratio.

Analogous to coexistence in other communities based on essential resources (Vincent et al., 1996), consumer coexistence is based on tradeoffs in elemental conversion efficiencies.

A study by Behmer and Joern (2008) provides anecdotal support for a community based on essential resources. In a grasshopper community of seven *Melanoplus* species, each grasshopper had a unique intake target of carbohydrate and protein. By raising each species on single diets consisting of different ratios of carbohydrates to proteins, and measuring growth rate and developmental time, Behmer and Joern found evidence that these diet-selection behaviors are adaptive. The authors conclude that the grasshoppers' divergent diet-selection behaviors may explain how such a large number of congeners can coexist via "stoichiometric niche" partitioning. This is conceivable from the standpoint of our theory if there are as many resources as consumers and each consumer has a resource on which it performs best. Alternatively, as in Tilman's (1982) resource ratio theory, spatial heterogeneity in plant abundances may allow multiple consumers to coexist on just a few resources.

### **2.4.4** *Stoichiometric Constraints and Species Coexistence*

A corollary of our models' predictions is that a species' niche *sensu* Leibold (1995) may change dramatically based on resource equilibrium abundances, which are functions of resource harvest rates, conversion efficiencies, and consumer mortality. These variables, in turn, may differ as the habitat varies. For instance, a species' efficiency of converting resources may decrease with increasing latitude or elevation. Habitats may also impose different per-capita mortality rates. Thus, variation in

stoichiometric constraints at the landscape level could produce intriguing and complex community patterns.

Information about the nature of tradeoffs in elemental use efficiencies is essential to the application of this theory. Surprisingly, Passarge et al. (2006) found no tradeoffs in light-use efficiency versus phosphorus-use efficiency among five quite-different phytoplankton species. If tradeoffs in elemental use efficiencies do not exist, then our model predicts that consumers cannot coexist when resource equilibrium abundances are relatively high. However, consumers can still coexist when resource equilibrium abundances are relatively low, provided the appropriate tradeoff exists. Furthermore, consumer tradeoffs in both resource encounter rates and elemental conversion efficiencies result in different communities than those produced from tradeoffs in either of these traits alone. Thus, understanding fitness tradeoffs between elements, and when co-adaptations of elemental use efficiency with other resource garnering traits should occur, will help to elucidate the role of stoichiometry in generating diversity and structuring communities.

### **2.4.5** *Conclusion*

Our theory highlights how foraging behavior impacts species coexistence (stability of equilibrium points) and species resource requirements (shapes of ZNGIs). Competing consumers coexist only if appropriate fitness tradeoffs are present between them. Our theory shows how different tradeoff assumptions change the outcomes of community structure. On a final note, we propose that a consumer-resource modeling approach such as ours can provide a framework for better integrating physiological and

community ecology. In the context of the population, physiology usually imposes a constraint on consumer fitness. This fact alone is not particularly interesting, but considering this constraint within a dynamic environment, in the context of feedbacks between the environment, fitness, foraging behavior and species interactions, should lead to the development of more ecologically insightful models.

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# 3. Switching Strategies, Population Dynamics, and Mechanisms of Coexistence in Food Webs with Jekyll-and-Hyde Species

## 3.1 Introduction

Predation can promote the coexistence of two consumer species competing for a common resource. This occurs in one of two ways. First, a distinct predator species preys more successfully or heavily on the consumer species that is itself the better competitor in the absence of predation (Paine 1966, Leibold 1996, Viola et al. 2010). Or, secondly, via intra-guild predation, the poorer resource competitor is able to prey upon the other consumer species (Rosenzweig 1966, Holt and Polis 1997, Borer et al. 2003). With intra-guild predation, one of the consumers incidentally or intentionally preys upon the other consumer. Intra-guild predation becomes common in nature when the consumer's adaptations for its resource also make it reasonably apt at capturing the other consumer. For instance, the adaptations that permit a large spider species to capture herbivorous insects also allow it to capture smaller species of spiders. In fact, "mesopredators" often find themselves both competing with and being preyed upon by larger predators.

An important, but less appreciated, form of intra-guild predation occurs when the intra-guild predator species has two morphs: one competes with another consumer species; and the other preys upon it. We call the first morph Dr. Jekyll and the second Mr. Hyde after the story of the benign scientist who finds himself morphing into the malignant Mr. Hyde. Unlike the story, most ecological Jekyll-and-Hyde species possess a morph that cannot convert back within the lifetime of the individual. At some point in

its development or ecology, the individual irreversibly becomes the predatory morph. Only through offspring can the original morph or a mixture of morphs be recovered.

The single-cell ciliate, *Tetrahymena vorax,* represents a Jekyll-and-Hyde species. As a microstome, *T. vorax* feeds on bacteria, ingesting numerous individuals to form a food vacuole eventually. As a macrostome, *T. vorax* feeds on other ciliates in its guild (such as *T. pyriformis*), engorging itself through the capture of just a single prey item. An individual microstome metamorphoses into a macrostome by changing its morphology and the structure of its intake orifice. But it need not do so; a microstome can simply divide into a pair of macrostomes. The macrostome cannot morph back into a microstome. The microstome is an obligatory bacteria feeder, and the macrostome is an obligatory predator of other ciliates. They are Dr. Jekyll and Mr. Hyde, respectively. Morin (1999) examined competition between morphing and non-morphing ciliates and found that the non-morphing *Colpidium* outcompeted the morphing *Blepharisma* at low productivity. But at higher productivity, the two largely co-existed. Theory had predicted these outcomes.

Dr. Jekyll and Mr. Hyde morphs are present in other ciliates as well (e.g., *Lembadion bullinum*). They also occur, albeit infrequently, across the animal kingdom. The tiger salamander, *Ambystoma tigrinum nebulosum*, develops into a cannibalistic morph when macroinvertebrates are available to its larvae (Whiteman et al. 2003, Maret and Collins 1997). Both field and laboratory experiments show that cannibals prefer conspecifics over macroinvertebrates. On the other hand, typical morphs consume only macroinvertebrate and other prey and apparently never cannibalize conspecifics. Other trophic-induced polymorphisms, though not of a Jeckyll-and-Hyde nature, are common

among fish (e.g. many cichlid species, Meyer 1990; perch, Hjelm et al. 2001; charr, Garduño-Paz and Adams 2010). Wimberger (1994) and Skulason and Smith (1995) review trophic-induced polymorphisms among vertebrates.

Here we model the dynamics, switching strategies, and opportunities for species coexistence when the community contains a Jekyll-and-Hyde species, such as *T. vorax*. We consider both a constant and a variable switching strategy. We seek to answer three questions: 1) What is the effect of different switching strategies on population dynamics? 2) What is the effect of different switching strategies on coexistence with the intra-guild prey? 3) What is the relationship between switching species and their specialist counterparts? Jekyll-and-Hyde morphs represent an extreme form of prey switching that is more generally associated with specialist species rather than specialist morphs within a species. Therefore, we introduce non-switching specialists (that correspond to each morph) into the community to understand the relationship between a switching species and their specialist counterparts.

### 3.2 *Tetrahymena* as a Dr. Jekyll – Mr. Hyde system

The two main cell types of the polymorphic *T. vorax* are the microstomal form (77× 29 μm) with a characteristically small oral apparatus (10.5 × 5.5 μm) and the macrostomal form (110 × 75 μm) with a large oral apparatus (29 × 23 μm). In the microstome, a food vacuole forms as an individual continuously traps small particles including bacteria. In the macrostome, the large oral apparatus opens into a large, semi-permanent pouch (surface area = 5715 $\mu m^2$). The pouch acts as a prey receptacle prior to its pinching off from the cytostome.

The feeding strategies of the microstome and macrostome cells differ radically, reflecting their prey. In the filter-feeding microstome, ciliary membranelles produce a current that sweeps bacteria and other small particles across the oral apparatus in which they are trapped by the cilia of the undulatory membrane (which acts as baleen allowing water to pass through). The trapped particles then fall down the oral ribs into the cytostome where phagosomes form.

In contrast, the macrostome ingests small ciliates whole as it moves through a liquid medium with its cytostomal opening partially agape. When a prey item passes through the cytostome, the macrostome accelerates, forcing the prey into the pouch. The pouch then seals off from the cytostome forming a large phagosome in which digestion occurs. Following phagosome formation, a new pouch forms.

The two feeding strategies are cell type specific because of the relative sizes and morphologies of their respective feeding structures. The microstomal form cannot feed on large prey and the macrostomal form cannot feed on small prey.

A potential prey ciliate also produces the trigger for microstomal to macrostomal differentiation. Buhse (1966a, 1966b, 1967) isolated a mixture of cell exudates as the triggering factor and named it "stomatin" because it initiated the differentiation of the macrostome from the microstome. More recently Ryals et al. (2002) identified the active principle as an iron chelate of hypoxanthine and uracil. The chelate apparently forms as part of an elimination process of purine and pyrimidine catabolites. The chelate may bind to a receptor, which activates genes that initiate the differentiation process.

Differentiation of the macrostome occurs by replacement of the microstomal oral apparatus. The apparatus is resorbed and replaced with a larger one typical of the

macrostomal form. The process ends with formation of a prey receptacle called the cytopharingeal pouch (Buhse 1966b). The differentiation process occurs in 6 to 8 h following treatment with stomatin or the chelate. Maximal differentiation occurs when populations of microstomal cells are in the early, stationary phase of population growth (Buhse 1966a) and most cells appear in the late S or G2 stage of the cell cycle.

The macrostome is a transient condition contingent upon prey availability and apparently correlated with cell division. If ciliate prey are abundant, macrostome cell division produces macrostomes. If ciliate prey are scarce, macrostome cell division produces microstomes (Buhse 1966a). Switching may be adaptively modulated in response to feeding opportunities provided by bacteria (to the microstome) and other ciliates (to the macrostome).

### 3.3 *Model Description*

We consider two different food webs: 1) a web without cannibalism, where the Hyde morph feeds solely on the intraguild prey species, and 2) a web with cannibalism, where the Hyde morph feeds on both the intraguild prey and the Jekyll morph (Figure 3.1). In our analysis of these models, we consider a resource species, intraguild prey, and a switching species with two distinct morphs. Furthermore, in order to understand the effect of switching on population dynamics, opportunities for coexistence, and the adaptive value of switching, we also consider two specialist species corresponding to each of the morphs. While ecologically identical to the switching morphs, they are distinct species that do not switch.

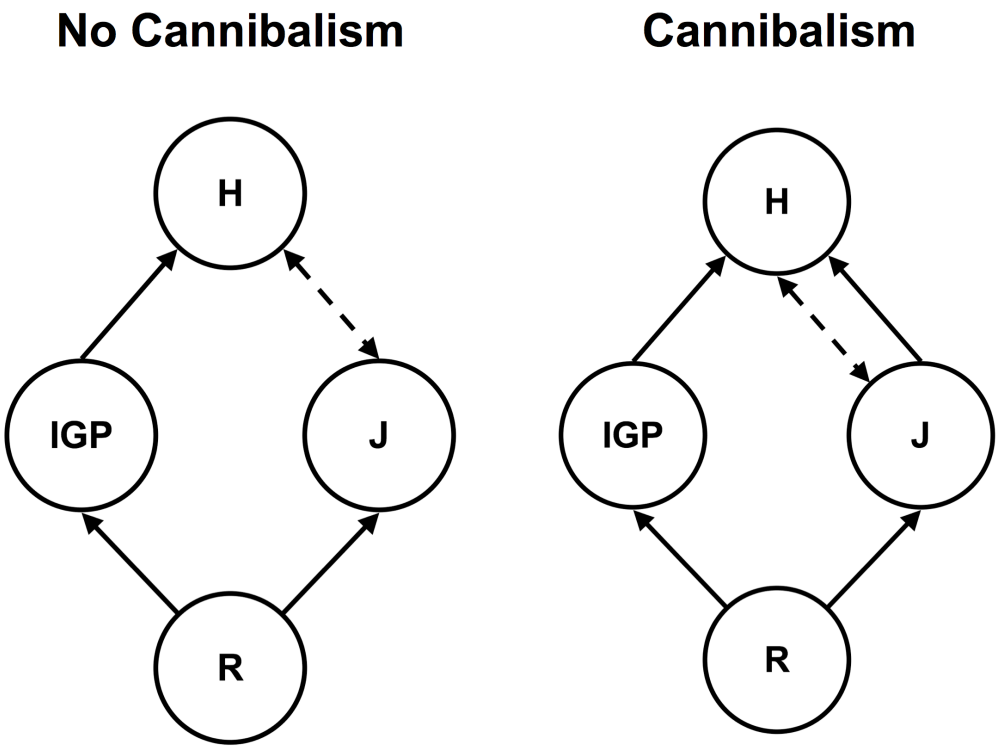


**Figure 3.1:** Graphical depiction of the food webs we model. The solid arrows indicate trophic transfers. The dotted arrows indicate switching between the morphs of the polymorphic species. H, J, IGP, and R stand for Hyde, Jekyll, intraguild prey, and a resource species, respectively.

We build upon the MacArthur (1972) consumer-resource model (Table 3.1). At the base of each web is a resource species, which is self-limited by logistic growth. Consumers have type-II functional responses (Holling 1959) and suffer constant density independent mortality. Intraguild predation is introduced through the linkage of Jekyll and Hyde morphs as a single switching species. The variables $c_j$ and $c_h$ are the per capita switching rates of Jekyll to Hyde and Hyde to Jekyll, respectively. We model two different switching strategies – a constant per capita switching rate, and a variable

switching rate. When switching rate is variable, we let the rate of switching increase with the fitness difference between the potential morph and the individual's current morph. This is analogous to switching models from habitat selection. Throughout, we assume for simplicity that switching is cost free.

To model variable switching we use a β-function (e.g. Fryxell and Lundberg, 1998, Equation 1). We let our model organism estimate the fitnesses of each morph from environmental information. Fitness of a morph is defined as its per capita growth rate in the absence of those switching into and out of the morph. The switching function is parameterized by the switch point ($\delta$) and the sensitivity of switching to changes in the environment ($z$). By setting the switch point $\delta$ to 0, the morphs are equally likely switch when there is no fitness difference between the morphs. Individuals using this strategy become more likely to switch morphs when the alternate morph has higher fitness than the morph they currently occupy. Perfectly adaptive behavior occurs when $z$ approaches infinity in the model.

$$c_k = \frac{e^{z\delta}}{e^{z\delta} + e^{zx}} \tag{1}$$

In the following sections, we first investigate the effects of the switching strategies on population dynamics and coexistence with intraguild prey. Then we consider the relationship of switching species and their specialist counterparts. We address these questions mainly through numerical simulation of the model. We begin by choosing parameters that allow both specialist species to coexist with the intraguild prey within a range of environmental productivities ($K$, Table 3.2). Following Holt and Polis (1997), we assume a predation risk-foraging tradeoff between Jekyll and the intraguild prey species, such that Jekyll is better at managing predation risk and the intraguild prey

is a superior resource exploiter. Then we vary select model parameters in order to explore the generality of our conclusions. Throughout we compare and contrast the two food webs and the two switching behaviors.

**Table 3.1:** Equations governing the dynamics of the food webs.

| Description | Food web without cannibalism | Food web with cannibalism |
|---|---|---|
| Resource growth rate | $\frac{dR}{dt} = R\left[r\left(1-\frac{R}{K}\right) - \frac{a_{rp}P}{1+a_{rp}h_{rp}R} - \frac{a_{rj}\sum_{i=1}^{2}J_i}{1+a_{rj}h_{rj}R}\right]$ | $\frac{dR}{dt} = R\left[r\left(1-\frac{R}{K}\right) - \frac{a_{rp}P}{1+a_{rp}h_{rp}R} - \frac{a_{rj}\sum_{i=1}^{2}J_i}{1+a_{rj}h_{rj}R}\right]$ |
| Intraguild prey growth rate | $\frac{dP}{dt} = P\left[\frac{b_{rp}a_{rp}R}{1+a_{rp}h_{rp}R} - m_p - \frac{a_{ph}\sum_{i=1}^{2}H_i}{1+a_{ph}h_{ph}P}\right]$ | $\frac{dP}{dt} = P\left[\frac{b_{rp}a_{rp}R}{1+a_{rp}h_{rp}R} - m_p - \frac{a_{ph}\sum_{i=1}^{2}H_i}{1+a_{ph}h_{ph}P+a_{jh}h_{jh}\sum_{i=1}^{2}J_i}\right]$ |
| Jekyll growth rate | $\frac{dJ_i}{dt} = J_i\left[\frac{b_{rj}a_{rj}R}{1+a_{rj}h_{rj}R} - m_j - c_{ji}\right] + c_{hi}H_i$ | $\frac{dJ_i}{dt} = J_i\left[\frac{b_{rj}a_{rj}R}{1+a_{rj}h_{rj}R} - m_j - c_{ji} - \frac{a_{jh}\sum_{i=1}^{2}H_i}{1+a_{ph}h_{ph}P+a_{jh}h_{jh}\sum_{i=1}^{2}J_i}\right] + c_{hi}H_i$ |
| Hyde growth rate | $\frac{dH_i}{dt} = H_i\left[\frac{b_{ph}a_{ph}P}{1+a_{ph}h_{ph}P} - m_h - c_{hi}\right] + c_{ji}J_i$ | $\frac{dH_i}{dt} = H_i\left[\frac{b_{ph}a_{ph}P+b_{jh}a_{jh}\sum_{i=1}^{2}J_i}{1+a_{ph}h_{ph}P+a_{jh}h_{jh}\sum_{i=1}^{2}J_i} - m_h - c_{hi}\right] + c_{ji}J_i$ |

*Note*: The *i* subscripts on both Jekyll and Hyde and their switching rates indicate either a single morph of the switching species or a separate specialist species. The sums then represent the abundance of both the morph and the specialist species, since they are ecologically equivalent in all respects besides switching strategy.

**Table 3.2:** Parameter meanings and values used in simulations.

| Parameter | Meaning | Value (units) |
|---|---|---|
| $K$ | Resource carrying capacity | Variable (resource biomass) |
| $r$ | Resource intrinsic growth rate | 1 ($time^{-1}$) |
| $a_{rj}$ | Encounter rate of Jekyll on resource | 0.075 ($time^{-1}$) |
| $a_{rp}$ | Encounter rate of intraguild prey on resource | 0.1 ($time^{-1}$) |
| $a_{ph}$ | Encounter rate of Hyde on intraguild prey | 0.01 ($time^{-1}$) |
| $a_{jh}$* | Encounter rate of Hyde on Jekyll | 0.005 ($time^{-1}$) |
| $h_{rj}$ | Handling time of Jekyll on resource | 0.1 (time/resource) |
| $h_{rp}$ | Handling time of intraguild prey on resource | 0.1 (time/resource) |
| $h_{ph}$ | Handling time of Hyde on intraguild prey | 2 (time/Intraguild Prey) |
| $h_{jh}$* | Handling time of Hyde on Jekyll | 2 (time/ Jekyll) |
| $b_{rj}$ | Conversion efficiency of captured resources into Jekyll | 0.05 (biomass Jekyll/ unit resource consumed) |
| $b_{rp}$ | Conversion efficiency of captured resources into intraguild prey | 0.05 (biomass of Intraguild Prey/ unit resource consumed) |
| $b_{ph}$ | Conversion efficiency of captured intraguild prey into Hyde | 0.15 (biomass of Hyde/unit Intraguild Prey consumed) |
| $b_{jh}$* | Conversion efficiency of captured Jekyll into Hyde | 0.05 (biomass of Hyde/ unit Jekyll consumed) |
| $m_p$ | Per capita mortality rate of intraguild prey | 0.05 ($time^{-1}$) |
| $m_j$ | Per capita mortality rate of Jekyll | 0.05 ($time^{-1}$) |
| $m_h$ | Per capita mortality rate of Hyde | Variable ($time^{-1}$) |
| $z$ | Switching sensitivity | Variable (unitless) |

* indicates parameters exclusive to the cannibal food web.

## 3.4 Results

### **3.4.1** *Switching strategies and population dynamics*

We begin by considering the different food webs and switching behaviors along a gradient of productivity ($K$). The top panel of Figure 3.2 shows the two food webs without switching. Here Jekyll and Hyde are two distinct specialist species. In these food webs there are three types of dynamical behavior. At low productivity the population dynamics are stable. At intermediate productivities the dynamics exhibit limit cycles. At higher productivities, the limit cycles have higher frequencies and amplitudes. Increasing $K$ causes the paradox of enrichment (Rosenzweig 1971), i.e., increasing productivity destabilizes the dynamics.

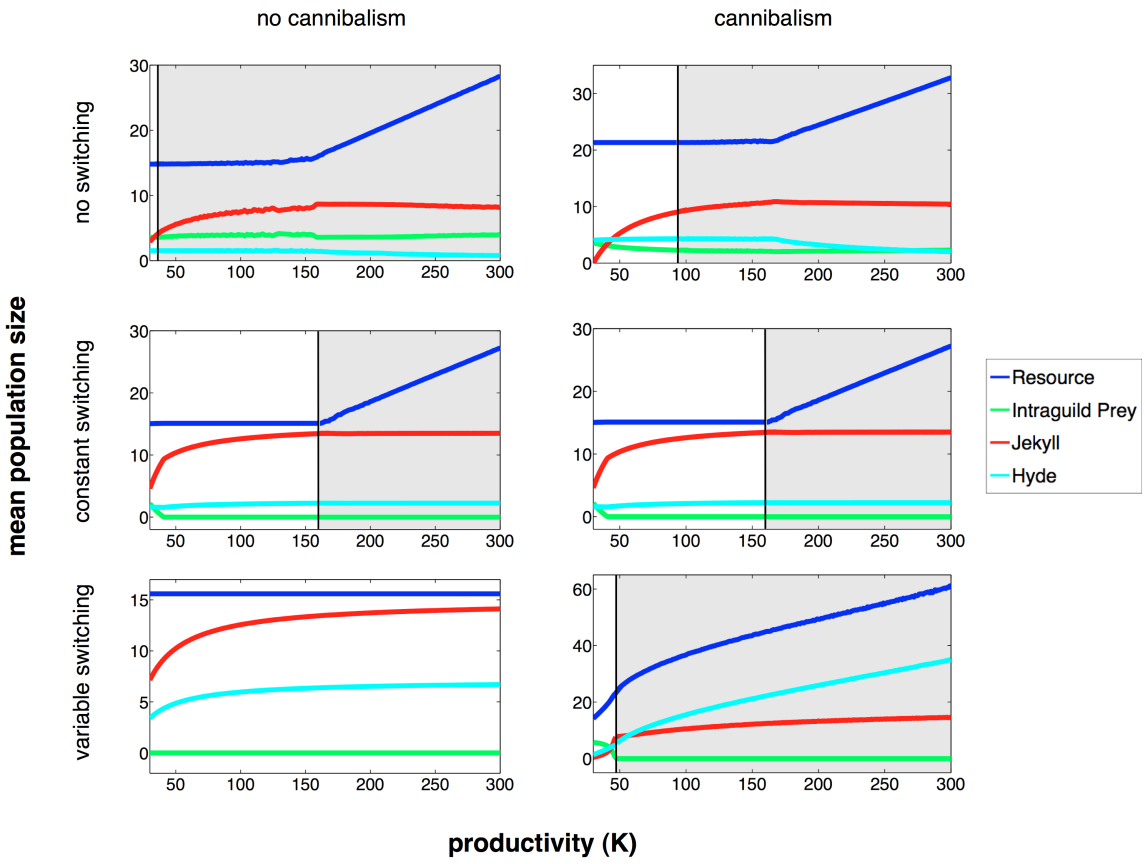


**Figure 3.2:** Population stability and species coexistence as a function of productivity ($K$) for the two food webs, with no ($c = 0$), constant ($c = 0.001$), and variable ($z = 100$) switching strategies. Population values shown are means of simulated time series after the dynamics have reached equilibrium. Shaded and un-shaded areas indicate population dynamics characterized by limit cycles and stability respectively.

The middle panels of Figure 3.2 show the two food webs with a fixed and a low rate of switching ($c = 0.001$). The constant switching behavior stabilizes the population dynamics. In both models, the limit cycles do not occur until very high productivities. The stabilizing effect of switching comes about through increased negative density dependence. To demonstrate this, we let $\gamma$ equal the net per capita rate of transfer from Hyde to Jekyll. $\gamma = -c + c\frac{J}{H}$. Taking the derivative of $\gamma$ with respect to $H$ gives the

direct per capita density dependence due to switching. $\frac{\partial \gamma}{\partial H} = -\frac{cJ}{H^2}$. Thus switching adds negative density dependence, which shortens time lags and stabilizes the dynamics. The effect is strongest when $H$ (Hyde abundance) is small. This happens because the per capita outflow from a particular morph is constant, but the per capita inflow decreases with morph population size.

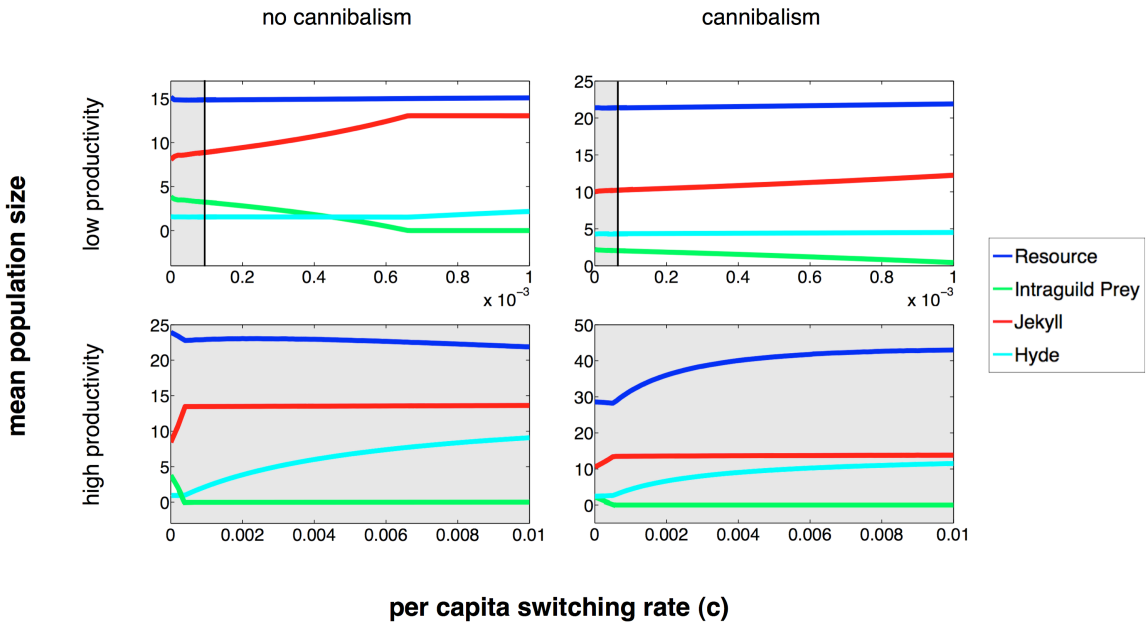


**Figure 3.3:** Population stability and species coexistence as a function of the per capita switching rate ($c$) of constant switchers in the two food webs under low ($K$ = 125) and high ($K$ = 250) productivity. Population values shown are means of simulated time series after the dynamics have reached equilibrium. Shaded and un-shaded regions indicate population dynamics characterized by limit cycles and stability respectively.

Figure 3.2 shows the effects of increased per capita rate of constant switching ($c$). These results confirm and reinforce the conjectures that switching is stabilizing. Under low productivity, increasing the switching rate quickly stabilizes the dynamics. Under high productivity, the dynamics are not stabilized for the range of switching rates shown. We note that, in both food webs the dynamics do stabilize by $c = 0.1$.

The variable switching strategy has different consequences for stability depending upon food web structure. In the non-cannibal food web, variable switching (bottom panel of Figure 3.2) is more stabilizing than the constant switching strategy. Large increases in a morph's population result in negative fitness (per capita growth rate excluding switching), which result in increased per capita switching outflows from the morph. In the non-cannibal web, the dynamics are stabilized over a wider range of productivities. In the cannibal web, however, variable switching is actually less stabilizing than constant switching. Since Hyde feeds on Jekyll, predator-prey dynamics coupled with the variable switching result in cannibalistic population cycles. The positive feedback in Hyde abundance drives this instability. Increases in Hyde abundance immediately decreases Jekyll's fitness, which in turn causes Jekyll to switch to Hyde, further inflating Hyde abundance.

### **3.4.2** *Switching strategies and coexistence with intraguild prey*

For the parameters used in Figure 3.2, constant switching restricts the conditions under which Jekyll, Hyde, and the intraguild prey coexist. To understand why, consider the effects of productivity on the two food webs. In the non-cannibalistic food web, increasing $K$ allows the resource to support more consumers. Since Hyde fixes the

abundance of intraguild prey, and Jekyll fixes the abundance of the resource, increasing *K* translates into more Jekyll. When the two morphs are linked through switching, the increase in Jekyll increases Hyde to an artificially high level. As a result Hyde depletes the intraguild prey. Therefore, increasing productivity combined with switching increases the per capita mortality rates on the intraguild prey, and decreases opportunities for coexistence.

In the cannibalistic food web, Hyde feeds on both Jekyll and the intraguild prey as substitutable resources. The negative effect of subsidized Hyde abundance still occurs. However, since the intraguild prey and Jekyll are both part of Hyde's functional response, there is also an indirect mutualistic effect between the intraguild prey and Jekyll. Increasing Jekyll causes Hyde individuals to spend more time handling them, which then decreases predation pressure on the intraguild prey. This counteracts the effects of subsidies to Hyde. This short-term mutualistic effect between Jekyll and intraguild prey in the cannibalistic web enhances coexistence of the intraguild prey relative to the non-cannibalistic web. But coexistence is still limited relative to the cannibal web with no switching.

Figure 3.3 shows how increased switching rates drive the intraguild prey extinct. This is because Jekyll subsidizes the abundance of Hyde. This subsidy grows as the switching rate increases, thus amplifying this effect. In addition, the effect is also enhanced by productivity. Under high productivities, the intraguild prey goes extinct at comparatively lower switching rates. At higher productivities the system fluctuates at high frequency and greater amplitude. These types of fluctuations increase the benefit of higher switching rates. As the switching rates increase, the switching species becomes

more efficient and both bacteria and intraguild prey abundances are decreased. This increased efficiency drives the intraguild prey extinct in both food webs.

Interestingly, we found that intraguild prey coexistence depends upon the relative efficiencies of Jekyll and Hyde - i.e. which morph is subsidized and which is subsidizing. The morph with the higher average abundance is the morph that acts as a subsidy, since the per capita rate of switching is equal in both directions between the morphs. When Jekyll subsidizes Hyde abundance (as is generally the case in Figure 3.2), coexistence becomes restricted relative to the food web with no switching. Alternatively, when Hyde subsidizes Jekyll abundance, coexistence is enhanced. This occurs because the intraguild prey is more limited by predation than food. If Hyde subsidizes Jekyll abundance, predation pressure on the intraguild prey decreases and the shared resource is depressed. Since the intraguild prey is more limited by predation, an increased switching rate will temporarily increase the fitness of the intraguild prey.

The variable switching strategy greatly reduces the possibility of coexistence with the intraguild prey. The intraguild prey coexists only at very low productivity (bottom panel of Figure 3.2). In the specialist web, the variable switching is stabilizing, and at equilibrium, switching rates are high. This increases subsidies between morphs and drives the intraguild prey extinct. In the cannibal web, switching-driven cannibalistic cycles inflate the average abundance of Hyde and drive the intraguild prey extinct.

### **3.4.3** *Switching versus specializiation*

In this section we investigate the relationship between a switching species and its specialist counterparts. When do they coexist and when does one outcompete the other?

To answer these questions we expand the food webs to include both the switching species and its specialist counterparts.

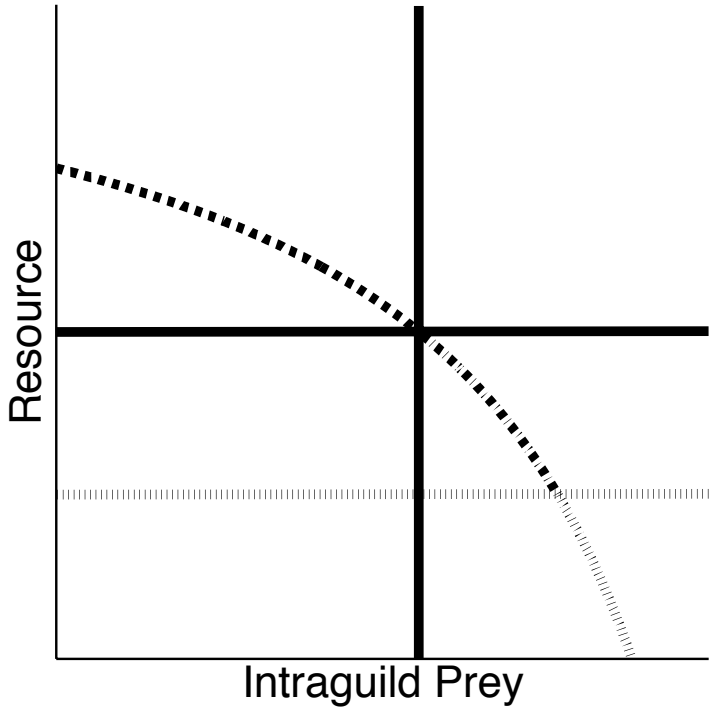


**Figure 3.4:** Zero net growth isoclines of the two specialist species (solid lines) and a constant switching species (dashed line) in the non-cannibalistic food web. The solid vertical line is the ZNGI of the Hyde specialist. The solid horizontal line is the ZNGI of the Jekyll specialist. The dotted horizontal line is the ZNGI of the intraguild prey when it suffers no mortality from predation. The switcher's ZNGI is broken into three regions. Equilibrium points on the dashed region ensure that Jekyll subsidizes Hyde. Equilibrium points on the dash-dot portions of the ZNGI ensure that Hyde subsidizes Jekyll. The dotted portion of the switcher's ZNGI represents a region where no equilibrium points can occur, since the intraguild prey will be driven extinct.

Figure 3.4 shows the Zero Net Growth Isoclines (ZNGIs) of the specialist species and the switching species (the appendix derives the ZNGIs) with constant switching in the non-cannibalistic food web. The ZNGIs represent all combinations of resource

abundance and intraguild prey required for a given species to subsist (Tilman 1980). The relative simplicity of the non-cannibalistic food web allows us to derive this graphical description of the model. On the other hand, the multidimensionality of the switcher's growth function in the cannibalistic web requires a four-dimensional ZNGI. However, the general results illustrated by the ZNGIs of the non-cannibalistic web also apply to the cannibalistic web.

In simple communities including the resource, intraguild prey, and the switching species, equilibrium points could occur at any point on the switcher's ZNGI (excluding the dotted portion of the switcher's ZNGI). Under these circumstances, one morph will be subsidizing the other. For instance, if the equilibrium point occurs on the dashed portion of the switcher's ZNGI, resource abundance is higher than the requirements of the Jekyll specialist, and intraguild prey abundances are lower than the requirements of the Hyde specialist. Therefore, the Jekyll morph subsidizes Hyde. Alternatively, equilibrium points on the dash-dot portion of the switcher's ZNGI result in Hyde subsidizing Jekyll. Note that equilibrium points cannot occur on the dotted portion of the switcher's ZNGI because such points will drive the intraguild prey extinct. As a consequence of these inherent subsidies, the specialist species corresponding to the subsidizing morph can always invade. In fact, there is an equilibrium point where the switcher and the two specialist species can all coexist. This is a consequence of having no cost to switching.

To study competition and coexistence between the switching species and its specialist counterparts, we varied the efficiencies of both Jekyll and Hyde by varying their per capita mortality rates. We then determined stable community outcomes. The

different food web structures and switching strategies interact to produce different regions of community outcomes (Figure 5). Outcomes depend upon both the stability and direction of subsidies between the morphs. Limit cycles produced by the specialist species can be sustained when a switching species is present. Given limit cycles, the switcher may coexist with the specialist corresponding to the subsidizing morph (regions C and D). Limit cycles can also create a situation where the switching species outcompetes both specialists (region B). However, this occurs only in very narrow regions of parameter space where the relative efficiencies of Jekyll and Hyde are nearly equal such that subsidies from one morph to the other are effectively absent. Stable dynamics can result in the coexistence of the switching species with both specialist species (region E).

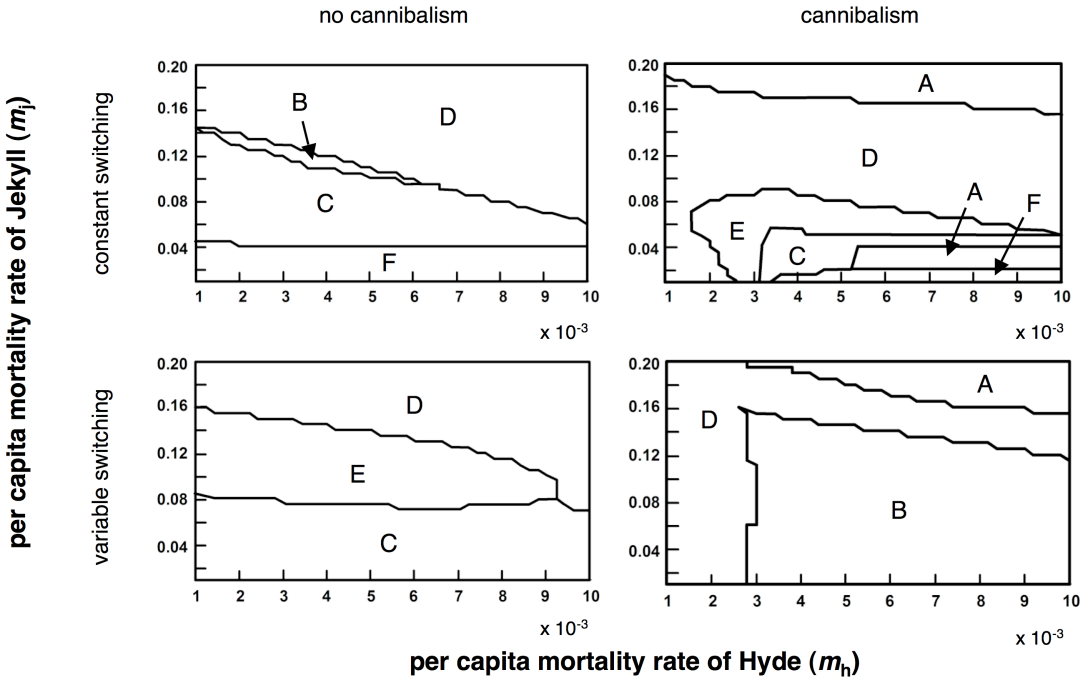


**Figure 3.5:** Species coexistence as a function of the per capita mortality rates of Jekyll ($m_j$) and Hyde ($m_h$) in the two food webs with constant ($c$ = 0.001) and variable ($z$ = 100) switching strategies. Letters differentiate community outcomes. A – The switching species is driven extinct. B – The switching species is present and both specialists are driven extinct. C – The switching species and the Jekyll specialist coexist. D – The switching species and the Hyde specialist coexist. E – All species coexist. F – No numerical solution found. For these simulations $K$=250.

Figure 3.5 shows that when the Jekyll morph is relatively more efficient, it subsidizes Hyde, and the Jekyll specialist can coexist with the switcher (region C). The coexistence of the Hyde specialist (region D), however, depends on food web type. In the non-cannibalistic food web, the Hyde specialist coexists over a broader range of Jekyll efficiencies when Hyde efficiency is lower. This counterintuitive result occurs because in the non-cannibal web, increases in Hyde mortality indirectly decrease

equilibrium abundances of Jekyll. For constant switchers, as long as Hyde abundances are on average greater than Jekyll, Hyde will be subsidizing Jekyll. In the cannibalistic food web, the Hyde specialist coexists over a broad range of Jekyll efficiencies when Hyde efficiency is higher. In both the non-cannibalistic web with variable switching and the cannibal web with constant switching, there is a region where the dynamics are stabilized and all species coexist (region E). In the cannibal webs, there is a region where the switching species is driven extinct (A). When Jekyll has low enough efficiency, the Jekyll specialist goes extinct and the Jekyll morph is strongly subsidized by the Hyde morph. Under these conditions, the Hyde specialist outcompetes the switcher and coexists with the intraguild prey.

The cannibal web with variable switching is distinct. For most of the parameter space, the switching species drives both specialists extinct (region B). In this region, the switching species displays switching-driven cannibalistic population cycles. Both Jekyll and Hyde have negative fitnesses over the long-term course of these cycles. As a result, when rare, neither specialist species can invade the system. The variable switching strategy permits the Jekyll and Hyde morphs of the switching species to exploit favorable times and avoid unfavorable times.

**3.5 Discussion**

Our investigation of the ecological behavior of communities with polymorphic intraguild predators, complements a body of empirical work regarding trophic polymorphisms (e.g. Skulason and Smith 1995, Banjeri and Morin 2009). We found three key results: 1) Switching stabilizes population dynamics. 2) Switching can either enhance or restrict opportunities for coexistence with intraguild prey species depending

on which morph is subsidizing. 3) Subsidies between the two morphs of the switching species promotes coexistence with a specialist species.

A species that can switch between trophic levels stabilizes population dynamics by increasing negative density dependence within each morph. However, our model assumes instantaneous switching. Time lags in the switching process might be destabilizing. In *T. vorax*, switching takes an average of 4.5 hours (Buhse 1966a), approximately half that of their generation time of 8 hours (Williams 1961). Moreover, predatory Hyde morphs are generally larger than their Jekyll counterparts, and switching may require cell division or lengthy developmental changes (e.g. Whiteman et al. 2003, Banerji and Morin 2009). These details of phenotypic switching will ultimately influence stability. Our model also shows how the feeding behavior of the switching species influences stability. In the generalist food web with variable switching, cannibalism from the Hyde morph feeding on the Jekyll morph can induce cyclic population dynamics.

The question of stability is important because it has direct bearing on selection pressures favoring switching species. If there are costs to switching, in a stable system a switching species will not be selected for. More efficient specialist species can depress resource levels lower than that required by a switching species. A fluctuating system can select for switching species. Figure 3.3 shows how increased switching depresses resource abundances, demonstrating that switching can be evolutionarily advantageous in fluctuating environments. It also shows how increased rates of switching stabilize the system. Thus, initially, mutant switchers may increase in abundance and even outcompete specialist phenotypes. However, the evolution of switching can stabilize the system and hence destroy the ecological conditions that selected for it. Therefore,

switching may need to evolve in systems where fluctuations are either driven by abiotic elements or other species in the system. Alternatively, cannibalism could drive the evolution of switching species. Interestingly, Loeb et al. (1994) found that tiger salamanders (*Ambystoma tigrinum nebulosum*) were induced to their cannibalistic morph only in the presence of conspecifics. However, their study did not include population dynamics.

In our model, switching can either enhance or restrict opportunities for coexistence with the intraguild prey. For the intraguild prey to coexist, it must be relatively more limited by predation than food. If Jekyll subsidizes Hyde, then switching Jekylls inflate Hyde abundances, thus increasing predation pressure on the intraguild prey and even further restricting opportunities for coexistence. The intraguild prey coexists only under low productivity. On the other hand, if Hyde subsidizes Jekyll, Hyde abundances will be depressed and predation pressure relieved. This in turn will enhance opportunities for coexistence, which can occur over a broad range of productivities. In traditional models of intraguild predation, the intraguild prey coexists if the intraguild predator is an efficient predator and poor competitor (Holt and Polis 1997). Alternatively, when predation acts more as a form of interference competition, intraguild predation can lead to alternate stable states. Moreover, Holt and Polis (1997) found that the intraguild prey tends to coexist under intermediate productivity levels.

Subsidies between the Jekyll and Hyde morphs decrease the average fitness of individuals of the switching species. This creates selective pressure for smarter switching strategies that use environmental cues to inform switching decisions. Variable switching rules are ubiquitous among known species exhibiting trophic polymorphism (e.g. protists

– Kusch and Hekmann 1992, fish – Garduño-Paz and Adams 2010, and salamanders – Loeb et al. 1994). For instance, *T. vorax* can be induced to switch from Jekyll to Hyde based on concentrations of an iron chelate released by ciliate prey (Ryals et al. 2002). Banerji and Morin (2009) have recently shown that switching in *T. vorax* is a coordinated adaptive response to environmental conditions. In our model, variable switching in the specialist food web is even more stabilizing than the constant switching strategy. In the cannibalistic food web variable switching combined with cannibalism caused population cycles. We also found that if switching is not perfect, then subsidies are reduced but still present.

Subsidies between the morphs create an ecological opportunity for the specialist species corresponding to the subsidizing morph. Generally, a single specialist species can coexist with the switching species. The exception occurs in the cannibal web with variable switching. In this case, over the majority of the parameter space, the switching species drove both of the specialists and the intraguild prey extinct. This was due to cannibalism driven population cycles. The switching species and both specialists coexisted only when the system exhibited stable population dynamics. However, given a cost to switching, the specialists should outcompete the switching species. Only under stable conditions are specialist species favored over switching species. Fluctuating conditions, with imperfect switching, allow a single specialist to coexist with the switching species. The realized niche of a switching species requires population fluctuations resulting from unstable dynamics or stochastic environments.

Skulason and Smith (1995) hypothesize that polymorphic species may be stepping-stones on the path to speciation. From this perspective, a switching Jekyll and

Hyde could be a transient species on its way to splitting into separate Jekyll and Hyde specialists. However, as we point out, a polymorphic species can occupy its own evolutionarily stable niche. Switching morphs is advantageous only if the environment at times favors one morph and at other times favors the other morph. Therefore, a switching species may occupy a stable peak on an adaptive landscape in a fluctuating environment. In this situation, the polymorphic traits are not correlated with ecological isolation, a condition necessary for speciation as hypothesized by Skulason and Smith (1995). Our model, therefore, suggests that only certain resource polymorphisms may in fact be stepping-stones to speciation.

*T. vorax* and other protist species provide opportunities to test the theory's predictions (Holyoak and Lawler 2005). Peter Morin's lab has extensively studied *T. vorax* (Price and Morin 2004, 2009, Banjeri and Morin 2009). Price and Morin (2004) found alternate stable states in systems with both *T. vorax* and another switching intraguild predator *Blepharisma americanum.* When *B. americanum* was initially present, *T. vorax* failed to invade the community. However, when *T. vorax* was initially present, *B. americanum* was able to invade, and the two species generally coexisted. In a subsequent study, Price and Morin (2009) found that relative initial densities of the two species did not directly cause the alternate stable states. Rather, the effect appeared to be mediated through bacterial depletion. Our theory does not address competition between intraguild predators, but could be modified to do so. However, our theory does highlight the importance of resource fluctuations to the success of a switching species. This could potentially play a role in determining the outcome of inter-specific interactions. In this case, how does each species influence stability and react to fluctuations in resources in

terms of phenotypic switching? Moreover, under what conditions does either morph of a species act as a subsidy? And how do these configurations influence the outcomes of inter-specific interactions?

In conclusion, our theory uncovers some of the main theoretical attributes of the community ecology of switching species. Interestingly, we found that although species exhibiting trophic polymorphisms have a broad niche breadth, they do not necessarily decrease the diversity of a system. They may actually provide ecological opportunities for specialist species. As Peter Morin's work highlights, aspects of this theory may be testable through the use of protist species in laboratory settings.

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# 4. Coadaptations of Feeding Behaviors and Gut Modulation as a Mechanism of Coexistence

## 4.1 Introduction

Heterogeneous foods seem to promote species diversity among consumers. We see frugivores, granivores, insectivores, herbivores and more. Often there are diet subdivisions among coexisting species within each of these broad diet categories. Between and within these categories, mechanisms of coexistence often seem to combine differences among the foods in nutritional composition with digestion tradeoffs. For instance, fruits offer simple relatively easy to digest carbohydrates in a bulky medium. Guts can be small with short throughput times. Insects offer a more nutritious but complex set of nutrients. Guts may be larger and throughput times longer, with numerous specialized enzymes. Seeds, leaves, grasses, lichens, earthworms, etc. all represent foods that may select for particular guts that may create niches for the coexistence of more or less specialized consumer species.

Foods can vary in energy reward, ease of handling, and in their bulk. When foods differ in these properties they can: 1) influence which food types to eat or not to eat (diet choice), 2) the size of the digestive tract and digestion rates (gut modulation with respect to gut volume and gut retention or throughput time), and 3) opportunities for the coexistence of different species selecting for or specializing on different foods. Here, we use evolutionary game theory within a consumer-resource framework to examine first, the coadaptations between diet choice, gut volume and throughput time through the gut. Second, we examine how the interplay between these coadaptations and the properties of

two different foods influences the evolution and coexistence of two consumer species on two food types.

In the absence of digestion limitations, a forager should rank food items based on energy to handling time ratio (*e/h*); and indeed most feeding animals have been shown to prefer foods with high *e*/*h* to those with low *e/h* (Stephens and Krebs 1986; Stephens et al. 2007). In fact, when *e/h* is sufficiently small a food ceases to be profitable and should not be included in the diet. When focusing on gut limitations, diet is also influenced by the bulk of the food and its digestible energy content (*e/b*). Either because of a "digestive pause" (Holling 1965) or because of gut filling (Jeschke et al. 2002), foods with higher energy to bulk ratios may be favored over those with lower ratios (Whelan and Brown 2005). The rate at which food clears the gut also becomes important for determining the extent to which gut capacity will limit or influence diets. By fermenting cellulose, ruminants such as deer, antelope, and cows have much longer throughput times than horses or other Perrisodactyls that pass large volumes of food quickly through the gut.

Recently, Whelan and Brown (2005) combined both the "external" and "internal" aspects of feeding into a model of optimal diet choice. By external, the forager had to search for and handle food items from its environment. By internal, the animal had capacity limitations of gut volume and passage rate that influenced time available for gathering food via gut fullness. Food preference in the form of *e/h* created the traditional boundary between whether a food should be rejected or accepted for consumption. Food richness (*e/b*) created a second boundary determining when some but not all of a particularly bulky food should be included in the diet, resulting in partially-selective diets. While feeding behavior was flexible, the gut volume and passage time were not.

Yet, many studies show how animals modulate these internal aspects of digestion in response to changes in diet (Starck and Wang 2005; Karasov and Martínez del Rio 2007).

Changes in diet substrate induce changes in gut structure and function. Whelan et al. (2007) modeled this by considering the optimal gut size and throughput time for a given food type that possessed properties of *e/h* and *e/b*. A larger gut, while more costly, reduced the length of the digestive pause associated with gut filling. A longer throughput time increased the food's digestibility at the expense of gut filling. The modeling considered foragers feeding on just one food type at a time (similar to many laboratory studies of gut modulation, such as Levey and Karasov 1992). With two foods there is the opportunity for a coadaptation among behavior (diet choice), gut volume and throughput time; and the opportunity for the evolution and coexistence of different consumer species with different suites of coadaptations.

Here we generalize and extend prior models by using a consumer-resource approach to predict the coadaptations between diet choice, gut volume and throughput time for a forager facing two different foods. When the two foods select for similar responses, then the forager should be able to accommodate both foods easily. When the foods select for very different gut volumes and/or throughput times, the abundances and characteristics of the two foods may challenge the forager to adopt a generalist gut. But, a forager with a generalist gut feeding opportunistically on both foods may not be evolutionarily stable (ESS), and may permit the invasion of species that have specialized guts and forage selectively on just one of the foods. Foods that place divergent demands on the gut may promote the coexistence of diet selectors. Following the extension of the model, we will determine which combinations of foods are likely to promote single

generalist species, or two more specialized species. And when there are two coexisting species, when will these be selective specialists, or when can we expect the coexistence of an opportunistic generalist with a selective specialist (Rosenzweig 1987, Brown 1990)?

**4.2 The Model**

The model presented here combines previous work on diet selection with gut constraints (Whelan and Brown 2005) and the evolution of gut modulation (Whelan et al 2000). We consider a consumer-resource game including n-consumers and 2-resources, where consumers evolve strategies that impact their efficiency of resource use through diet selection and gut modulation. The game theoretic component emerges because the consumers' strategies influence the abundance of resources, and the abundance of resources determines a consumer's fitness-maximizing strategy of behaviors and gut modulation.

In developing a consumer G-function (fitness generating function, Vincent and Brown 2005), we start with a consumer-resource model where salient parameters for the consumer include resource conversion efficiency, resource harvest rate, and consumer maintenance costs. Resource conversion efficiency for each resource is modeled using Michaelis-Menton kinetics:

$$e_i = \alpha_i e_{max,i}\, u_2/(\chi_i + \alpha_i u_2)$$

Here $\alpha_i$ is the rate (time$^{-1}$) that food i is digested (hydrolyzed) and then absorbed from the lumen of the small intestine (hereafter referred to simply as absorption rate), $e_{max,i}$ is the maximum resource conversion efficiency (reproductive biomass produced/unit resource consumed) for food i, $u_2$ is the time resources are processed

within the gut (throughput time), and $\chi_i$ is the half saturation constant for food i. Note that longer throughput times results in a higher assimilation of the potential nutrients contained in a food item. As throughput time gets very long, $e$ approaches $e_{max}$.

The harvest rate of a single resource is modeled using a modified Holling's disk equation (Holling 1959) that includes both external handling and internal processing of food:

$$H_i = (a_i y_i)/\{1 + a_i y_i[h_i + g_i m(B_i)]\}, \quad (1)$$

where $H_i$ is harvest rate, $a_i$ is encounter probability, and $y_i$ is resource abundance or density, for resource i. External handling, $h_i$, is identical to that in the original disc equation. Internal handling consists of two variables. The first, $g_i$, represents the actual processing of food within the gut, and the second, $m(B_i)$, represents the proportion of gut handling time that is exclusive of alternative foraging activities. External handling, $h$, and internal handling, $g$, have units of (time×item$^{-1}$).

Internal food processing, $g$, is determined by the quotient of food bulk per item, $b$ (ml×item$^{-1}$) and the volumetric flow rate of food through the gut, $Vo$ (ml×item$^{-1}$): $g = b/Vo$. But $Vo$ = gut capacity, $u_1$ (ml), divided by retention or throughput time, $u_2$ (time) [see Jumars and Martinez del Rio 1999]. Thus passage time per item is given by $g = (bu_2)/u_1$. Exclusive internal handling time, $m(B)$, increases monotonically with gut fullness. For simplicity, we let $m(B) = B$ (a linear function), the proportion of gut volume occupied by food. Gut fullness, $B$, is given by the sum of the bulk intake rate of both resources (bulk of the resource, $b_i$, multiplied by its ingestion or harvest rate, $H_i$) and the retention time of food in the gut (the quotient of throughput time, $u_2$, and gut volume, $u_1$): $B = (b_1H_1 + b_2H_2)(u_2/u_1)$. This definition of $m(B)$ allows the exclusivity of internal

handling to be a continuous, sliding scale that reflects the extent to which gut volume is filled from food consumption. Now let the probabilities of accepting resources 1 and 2 respectively be given by $u_3$ and $u_4$. Substituting $g_i$ and $B$ into (1), while allowing for a second resource, and simplifying yields

$$H_1 = (a_1 y_1 u_3) / [1 + a_1 y_1 u_3 (h_1 + b_1 (u_2^2/u_1^2)(b_1 H_1 + b_2 H_2) + a_2 y_2 u_4 (h_2 + b_2 (u_2^2/u_1^2)(b_1 H_1 + b_2 H_2)] \quad (2)$$

$$H_2 = (a_2 y_2 u_4) / [1 + a_1 y_1 u_3 (h_1 + b_1 (u_2^2/u_1^2)(b_1 H_1 + b_2 H_2) + a_2 y_2 u_4 (h_2 + b_2 (u_2^2/u_1^2)(b_1 H_1 + b_2 H_2)]$$

The harvest rates ($H_1$ and $H_2$) can be explicitly solved using the quadratic formula (see Whelan and Brown 2005).

Finally, the cost function ($\gamma$) includes both a fixed cost (for maintenance of all tissues including the gut) and a variable cost, which increases linearly with gut volume ($u_1$). The ease of resource harvest associated with large gut sizes is offset by the cost of maintaining a large gut:

$$\gamma = c + \beta u_1$$

Here $c$ is the fixed maintenance cost (time$^{-1}$), and $\beta$ is the variable cost of gut size (time$^{-1}$×gut size$^{-1}$).

Putting these components of fitness together we can construct the $G$-function for a consumer:

$$G = e_1 H_1 + e_2 H_2 - \gamma$$

The fitness generating function is equivalent to the per capita growth rate of the consumer, so the change in population growth rate is given by:

$$dx_j/dt = x_j G_j$$

For simplicity, we assume a linear relationship between net profit from foraging and per capita growth rate. Here $x_j$ is the population size of species j.

Resource population dynamics are given by a density-dependent renewal process minus the consumption by the consumer:

$$dy_i/dt = r_i(K_i - y_i) - x_i H_i$$

Here, $r_i$ is the maximum rate of resource renewal (time$^{-1}$), $K_i$ is the resource supply point, and $y_i$ is the population size of resource i.

### 4.3 Results

#### 4.3.1 Zero Net Growth Isoclines and Diet Selection Isolegs

The consumer's effect on the equilibrium abundance of resources and its diet selection can be displayed graphically with the use of Zero Net Growth Isoclines (ZNGI) and isolegs, respectively. ZNGIs are lines in the state space of resource abundances that give all combinations of $y_1$ and $y_2$ for which the consumer species' population growth rate is zero (Tilman 1982). The ZNGI is solved for by setting the consumer growth rate equal to zero and solving for $y_2$. The shapes of ZNGIs are influenced by the consumer's diet selection behavior and the attributes of the resources. Both determine the subsistence levels of resources. Isolegs also exist in this resource state space; they are curves that separate regions of resource abundances where the consumer has a different optimal feeding behavior (for isoleg solutions to this model see Whelan and Brown 2005).

The feature that separates this model from the classical model of diet selection is the internal handling time in the gut. In the absence of internal handling times, the model collapses to the classical diet selection model. In this situation, $e_i/h_i$ solely determines

food preferences, and only the Pulliam Isoleg (sensu Mitchell and Brown 1990) exists as a straight line (vertical or horizontal). It separates a region of complete selectivity on the better resource from a region of opportunism. The ZNGI is a negatively sloped straight line in areas of opportunism, and a vertical straight line in areas of selectivity (assuming $e_1/h_1 > e_2/h_2$).

Resources will differ in internal handling times when they differ in richness ($e_i/b_i$). Now, if we assume that resource 1 has greater richness and greater energy per external handling time, then the model produces a second isoleg. This isoleg, the Mitchell Isoleg (sensu Whelan and Brown 2005), separates a region of partial selectivity from a region of opportunism. The Pulliam Isoleg still exists but it now separates a region of complete selectivity from one of partial selectivity. Here, the ZNGI is bowed toward the origin (in a manner that produces slight complementarity between the two resources) in regions of opportunism, and a vertical straight line in regions of both partial and full selectivity (Fig. 1). The complementarity results from resource interactions via gut processing. When both foods 1 and 2 occur at low abundance, and the ratio of food 2 to food 1 ($y_2:y_1$) is low, the gut is mostly empty and the less rich resource, food 2, is valuable. As $y_2:y_1$ increases, the gut starts to become full from consuming food 2, and the value of food 2 declines as it's processing time-cost increases.

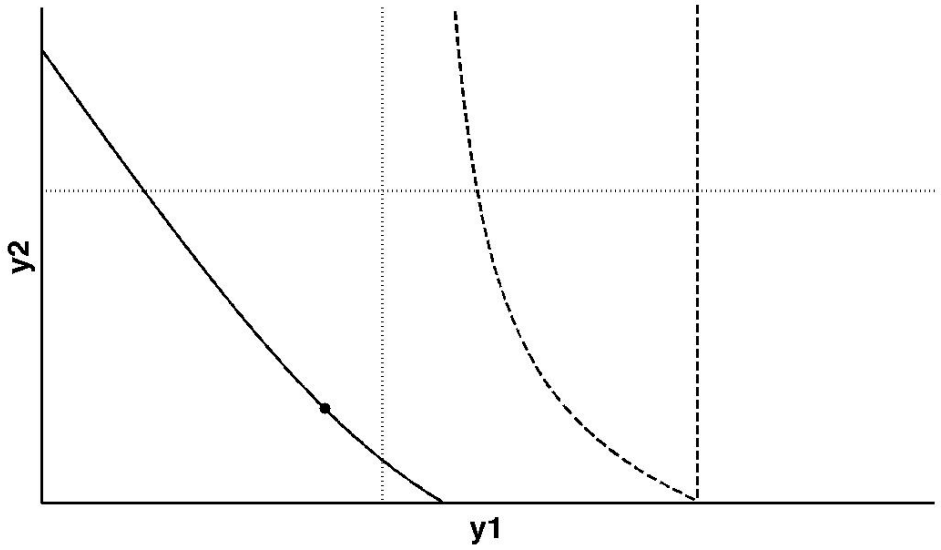


**Figure 4.1:** The effect of coadaptations of foraging behavior and gut physiology on the ZNGIs and behavioral isolegs of consumers feeding on two foods that differ only slightly in bulk. The resource state space of abundance of food 2 ($y_2$) versus the abundance of food 1 ($y_1$), contains three behavioral regions. To the right of the vertical dashed line, the Pulliam Isoleg, the consumer is selective on food 1. Between the Pulliam Isoleg and the curved dashed line, the Mitchell Isoleg, the consumer is partially-selective on food one, meaning it consumes some but not all of the encountered items of food 2. To the left of the Mitchell Isoleg the consumer harvests all encountered items of both foods opportunistically. The vertical and horizontal dotted lines represent the ZNGIs of specialist consumers that feed selectively on food 1 or food 2, respectively. The solid line with negative slope represents the ZNGI of the optimal strategy, a consumer with a generalist gut physiology. The dot represents the equilibrium abundances of foods 1 and 2 resulting from the consumption by the generalist species. Parameters for this figure are: $a_1 = a_2 = 0.1$; $h_1 = h_2 = 1.0$; $\alpha_1 = \alpha_2 = 0.1$; $e_{max1} = e_{max2} = 10$; $\chi_1 = \chi_2 = 1$; $b_1 = 1.3, b_2 = 2.7$; $c = 0.1$; $\beta = 0.1$; $r_1 = r_2 = 0.001$; $K_1 = K_2 = 100$.

### 4.3.2 One Resource: Evolution of Specialized Guts

Whelan et al. (2007) explored the adaption of guts specialized for particular food types. They considered a single consumer species depleting its single resource to a subsistence level (R* of Tilman, 1980). Their Figure 7 and Appendix Table 1 show how foods with different properties influence gut strategies (size and throughput time). We

begin our analyses by using our model to reanalyze Whelan et al.'s (2007). We found some discrepancies, which we have corrected in this paper's Table 4.1 and Figure 4.2. Numerical convergence to the optimum can be quite slow for some food types, and Whelan et al. (2007) appear to have stopped numerical analyses at strategies that performed close to optimal, but were still some distance from their ESS values.

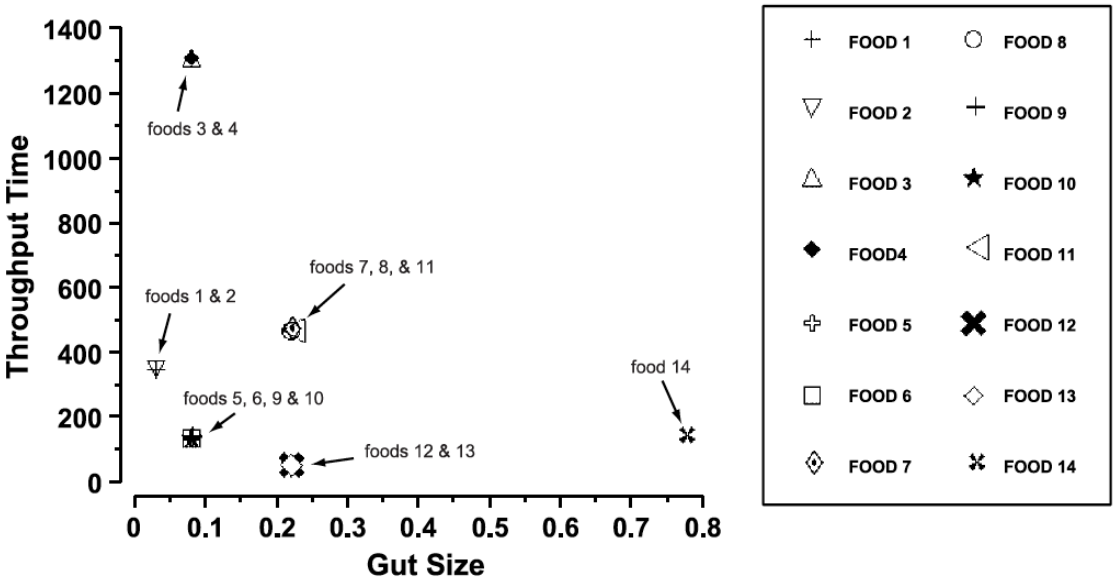


**Figure 4.2:** The effect of 14 different foods on the optimal gut size and throughput time. Foods differ in energetic reward, bulk, ease of absorption and external handling (see Table 1). Joint adjustment or modulation of gut size and throughput time results in six apparent digestive physiological syndromes. Increasing food richness (energy:bulk ratio) leads to smaller gut volumes and little change in throughput times. Higher absorption rates favor shorter throughput times with smaller effects on gut volume.

**Table 4.1:** Sixteen foods that differ in energetic value, **e**max, bulk, *b*, rate of absorption, *α*, and external handling time, *h*, and the optimal gut volume, *u1**, throughput time, ***u****2**, and minimal level of resource density, *y**, that result. The effective rate of absorption (**'*e*'**) and gut fullness are also shown when the forager has reached optimal adjustment of gut volume and throughput time at *y**.

| Food | $e_{max}$ | *b* | Richness (*e*/*b*) | α | *h* | $u_1^*$ (ml) | $u_2^*$ (time) | *y** | '*e*' (%) | Fullness (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 10 | 0.1 | 100 | 0.1 | 1 | 0.03 | 340 | 0.0011 | 97 | 12 |
| 2 | 10 | 0.1 | 100 | 0.1 | 10 | 0.03 | 340 | 0.0011 | 93 | 12 |
| 3 | 10 | 0.1 | 100 | 0.01 | 1 | 0.08 | 1300 | 0.0012 | 93 | 19 |
| 4 | 10 | 0.1 | 100 | 0.01 | 10 | 0.08 | 1300 | 0.0012 | 93 | 19 |
| 5 | 10 | 1 | 10 | 0.1 | 1 | 0.08 | 130 | 0.0012 | 93 | 19 |
| 6 | 10 | 1 | 10 | 0.1 | 10 | 0.08 | 130 | 0.0012 | 93 | 19 |
| 7 | 10 | 1 | 10 | 0.01 | 1 | 0.22 | 460 | 0.0016 | 82 | 31 |
| 8 | 10 | 1 | 10 | 0.01 | 10 | 0.22 | 460 | 0.0016 | 82 | 3 |
| 9 | 1 | 0.1 | 10 | 0.1 | 1 | 0.08 | 130 | 0.012 | 93 | 19 |
| 10 | 1 | 0.1 | 10 | 0.1 | 10 | 0.08 | 130 | 0.012 | 93 | 19 |
| 11 | 1 | 0.1 | 10 | 0.01 | 1 | 0.22 | 460 | 0.017 | 82 | 31 |
| 12 | 1 | 1 | 1 | 0.1 | 1 | 0.22 | 46 | 0.017 | 82 | 31 |
| 13 | 1 | 1 | 1 | 0.1 | 10 | 0.22 | 46 | 0.017 | 82 | 31 |
| 14 | 1 | 1 | 1 | 0.01 | 1 | 0.77 | 130 | 0.044 | 57 | 52 |
| 15 | 1 | 0.1 | 10 | 0.01 | 10 | 23.0 | 0 | — | — | — |
| 16 | 1 | 1 | 1 | 0.01 | 10 | 27.0 | 0 | — | — | — |

*Note:* Foods are roughly arrayed from most favorable (top) to least favorable (bottom). Note that two foods (15 and 16) will not sustain the forager. Note also that for each of the first 14 foods, the forager is food limited, but typically operates below peak rate of absorption (effective e < 100%) with a mostly empty gut.

The ESS of the consumer appears to balance the minimization of gut fullness with the maximization of resource conversion efficiency. For instance, a bulky food requires a large gut and a short throughput time. A food with a low absorption rate requires a large gut and a long throughput time. Intriguingly, most foods selected for strategies that left consumers with relatively empty guts and with throughput times far shorter than would confer maximum resource conversion efficiency (Table 4.1). Of interest is how external handling time, *h*, has little to no influence on the optimal gut strategies.

When inspecting Fig. 4.2, imagine three straight lines radiating from the origin in the state space of optimal gut size and throughput time. Each line represents a constant richness ($e_{max,i}/b_i$) of the resources. The line with the greatest slope represents resources with high richness (foods 1-4), the line with intermediate slope represents resources with intermediate richness (foods 5-11), and the line with the smallest slope represents resources with low richness (foods 12-14). Moving along each one of these equal richness lines away from the origin represents a decline in resource absorption rate ($\alpha$). Therefore, this figure depicts the effect of the interaction between resource richness and absorption rate on consumer gut evolution. We can see that as absorption rate, α, decreases, both gut volume and throughput time increase, but the relative changes in the two gut characteristics are set by the degree of resource richness. For example, when richness is high, lowering absorption rate slightly increases gut volume, but greatly increases throughput time. Since the resource is rich, by definition it is efficiently converted to new biomass and or takes up minimal room in the gut. Therefore, decreasing absorption rate selects for a longer throughput time while only slightly increasing gut size.

The reverse is true when richness is low. Resources with low richness by definition have low conversion efficiencies and/or high bulk. As absorption rate declines, the consumer responds by dramatically increasing gut size and slightly increasing throughput time. Resources low in absolute richness and absorption rate tend to quickly fill the gut as the optimal throughput time increases slightly. To accommodate all of this bulk, the optimal gut volume increases significantly.

To take a further step, we can analyze the subsistence level of resources, $y^*$'s, and gut evolution together (Table 4.1). Intuitively, resources with high absolute richness and absorption rate result in the lowest $y^*$'s, consumers require only a low standing crop to subsist. Resources with the lowest richness and absorption rate cause the highest $y^*$'s. The most efficiently used resources drive consumer evolution to the smallest gut sizes and intermediate throughput times, whereas the least efficient resources drive consumer evolution to large gut sizes and low throughput times. As examples, contrast Foods 1 and 2 versus Food 14 in Table 4.1.

In a more explicit example, we can focus on the instances where different foods (Foods 5, 6, 9 and 10 versus Food 14) select for the same throughput times but very different gut volumes (Fig. 4.2 and Table 4.1). The food selecting for the greater gut size, food 14, causes a greater $y^*$. Therefore, when throughput time remains the same, differences in the gut sizes of consumers specialized for different foods implies quantitative differences in these foods' properties.

**Table 4.2:** Evolutionary definitions for reward and richness.

| | **Reward** | **Richness** |
|---|---|---|
| **Absolute** | $e_{max,i}/h_i$ | $e_{max,i}/b_i$ |
| **Absorption** | $\alpha_I e_{max,i}/h_i$ | $\alpha_I e_{max,i}/b_i$ |
| **Effective** | $\alpha_I u_2 e_{max,i}/h_i(\chi_i + \alpha_I^* u_2)$ | $\alpha_I u_2 e_{max,i}/b_i(\chi_i + \alpha_I^* u_2)$ |

*Note*: In an evolutionary setting, resources acquire effective rewards and richnesses due to the effect of changing throughput time ($u_2$) on conversion efficiency ($e_i$).

When foods vary in absorption rate or richness and they select for different throughput times, then we need to clarify the definitions of reward and richness within an explicitly evolutionary context (Table 4.2). This is because throughput time and absorption rate combine to determine the effective amount of nutrients assimilated from a given food item. Whereas $e_{max}$ and $e_{max}/b$ are absolute rewards and absolute richness, respectively, they give rise to effective reward, $e$, and richness, $e/b$, when adjusted for the actual amount of nutrients assimilated by the gut. In Table 4.2, we also define and distinguish between absorption reward, $\alpha e_{max}$, and absorption richness, $\alpha e_{max}/b$ to make clear the value of a food item in terms of absorption per unit passage time. This gives us a new evolutionary perspective of how resources may be ranked by both reward and richness. Looking at the formula for effective richness, it is clear that changing gut volume alone will not alter the ranking of food preferences based on richness. Thus, when foods select for significantly different gut sizes (but not throughput times), we know that they lie along a quantitative niche axis.

We can now look at the reverse situation, where two foods require different throughput times, but the same gut sizes. Combinations of these foods lie vertically in state space of optimal gut strategies. There are two examples of such foods in Fig. 4.2, and upon examination of $y^*$'s (Table 4.1) we see that each pair has an equal $y^*$. Referring back to effective richness, we can confirm that the rank ordering of such foods depends upon the forager's throughput time. Each specialist would see its food as having a higher effective richness than the other food. Therefore foods that require different throughput times and equal gut sizes, lie along a purely qualitative niche axis. The distinction between whether pairs of food create quantitative versus qualitative niche axes

becomes critical for the evolution of consumer communities driven by coadapations of behavior and gut characteristics.

### 4.3.3 Two Resources: Numerical and Graphical Analysis

As our next step, we used Fig. 4.2 to select pairs of different foods that favor either similar or disparate gut strategies. We can then determine ESS solutions for the resulting two-resource system and see whether the ESS contains a single or two coexisting consumer species. We do this by examining both the adaptive landscapes (reveals evolutionary stability) and ZNGIs (reveals ecological stability of consumer-resource population dynamics). Ecologically, it is possible to have three consumer species that coexist (1 generalist species with 2 specialist species), but we never found these to be evolutionarily stable. ESS communities possessed either a single generalist species, a generalist coexisting with a specialist species, or two coexisting specialist species. We used Matlab to simultaneously solve for the ecological and evolutionary equilibria of the model. From the viewpoint of two coevolving specialists, resources can differ along a quantitative or a qualitative niche axis. In the following, we present examples of each and discuss how they determine community evolution.

**Table 4.3:** Combinations of parameters that affect digestion and simple relationships between them. k is a constant. Food combinations diverge along either a qualitative or quantitative niche axis.

| | $e_{max,i}$ | $\alpha_i$ | $b_i$ |
|---|---|---|---|
| $e_{max,i}$ | **Quantitative**<br>$e_{max,1} > e_{max,2}$ | **Quantitative**<br>$\alpha_i e_{max,i} = k$<br>$\alpha_2 > \alpha_1$<br>$e_{max,1} > e_{max,2}$ | **Quantitative**<br>$e_{max,i} b_i = k$<br>$e_{max,1} > e_{max,2}$<br>$b_2 > b_1$ |
| $\alpha_i$ | **Quantitative**<br>$\alpha_i / e_{max,i} = k$<br>$\alpha_1 > \alpha_2$<br>$e_{max,1} > e_{max,2}$ | **Quantitative**<br>$\alpha_1 > \alpha_2$ | **Quantitative**<br>$\alpha_i b_i = k$ |
| $b_i$ | **Quantitative**<br>$e_{max,i} / b_i = k$<br>$e_{max,1} > e_{max,2}$<br>$b_1 > b_2$ | **Qualitative**<br>$\alpha_i / b_i = k$<br>$\alpha_2 > \alpha_1$<br>$b_2 > b_1$ | **Quantitative**<br>$b_2 > b_1$ |

**Quantitative Differences in the Food:** When foods vary in bulk, $b$, energy reward, $e$, and/or absorption rate, $\alpha$, most will create quantitative differences between foods (Table 4.3). All pairs of foods that vary quantitatively produce similar results and community organizations. For example, consider two foods that differ only in bulk. We assume $b_1 < b_2$. Evolutionarily, food 1 is the better food, and this represents a quantitative niche axis in the sense that no matter what gut strategy a species possesses, food 1 will always be preferred. The specialist on food 1 (the less bulky resource) evolves a small gut and long throughput time. The specialist on food 2 (the more bulky resource) evolves a large gut and short throughput time. The optimal strategy for an opportunistic species is a generalist strategy that approximates some averaging of the extreme specialist strategies. A strategy of selectively feeding on food 2 and adopting the

corresponding food 2 specialist gut is never an ESS. Because food 1 remains preferred it is always optimal to accept all encountered items of food 1.

When the two foods differ only slightly in bulk, the ESS community is the generalist species (Fig. 4.1), the generalist gut does not compromise the forager's digestive aptitudes on the two foods and it has the advantage of offering the consumer considerably more food items to harvest. The food specialist that selectively feeds on food 1 cannot invade this community, as the generalist species depresses the abundance of food 1 to below the specialist's subsistence level (ZNGI).

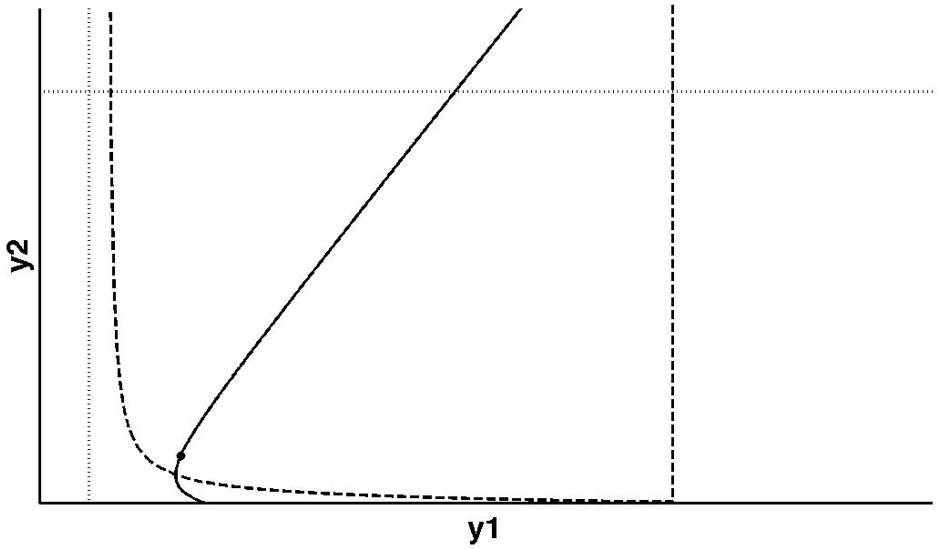


**Figure 4.3:** The effect of coadaptations of foraging behavior and gut physiology on the ZNGIs and behavioral isolegs of consumers feeding on two foods that differ greatly in bulk. The state space, and all lines except the solid line are the same as in Fig. 1. The solid line that switches from negative to positive slope represents the ZNGI of the optimal strategy, a consumer with a generalist gut physiology. The dot represents the equilibrium abundances of foods 1 and 2 resulting from consumption by the generalist strategy. In this figure, a specialist strategy on food 1 will out-compete the generalist strategy. Parameters for this figure are: $a_1 = a_2 = 0.1$; $h_1 = h_2 = 1.0$; $\alpha_1 = \alpha_2 = 0.1$; $e_{max1} = e_{max\,2} = 10$; $\chi_1 = \chi_2 = 1$; $b_1 = 0.1$, $b_2 = 3.9$; $c = 0.1$; $\beta = 0.1$; $r_1 = r_2 = 0.001$; $K_1 = K_2 = 100$.

As the bulk properties of the two foods diverge further, the ESS shifts from a single generalist species to an ESS with two species: a specialist on food 1 that feeds only on food 1; and a generalist species that feeds either opportunistically or partially selectively on food 1 (rejects some items of food 2 for harvest). These ESS strategies come about because a single species with the optimal compromise strategy of the generalist can no longer depress the level of food 1 sufficiently. A species that specializes and feeds selectively on food 1 can invade (Fig. 4.3). In fact, this specialist will outcompete the resident generalist species.

There are two ways to achieve the ESS of 2 species. 1) One can start with the specialist species and let the appropriate generalist invade. 2) Or, one can start with the single generalist species at its optimal (pre-invasion by the specialist) gut strategy. The specialist then invades causing a decline in the generalist's population size, an increase in the abundance of food 2, and a shift in its adaptive landscape towards a larger gut and shorter throughput time. If acclimation or adaptation along this landscape occurs fast enough then the generalist species can evolve into a region of its adaptive landscape where it can maintain a positive population size. At this point, the generalist continues to evolve towards the two species ESS, even as this species continues to consume food 1 and bulk up on food 2. Regardless of how the community gets to its ESS, the ESS remains the same.

The specialist species is evolutionarily unaffected by the generalist. The generalist does not influence the specialist's gut strategy nor its subsistence level of food 1. The generalist does influence the specialist's equilibrium population size, but not by as much as the specialist influences the generalist's population size. The presence of the

specialist strongly influences the generalist species and its evolution towards a larger gut and shorter passage rate.

**A Special Case:** A special case of the quantitative niche axis is when foods differ only in their sizes (Table 4.3, combination in lower left hand corner). This can be represented by foods that have the same absolute richness, but differ in their magnitudes of $e_{max}$ and $b$. If the energy per volume of the two foods is the same, then as their total volume changes ($b$), so does $e_{max}$. Since these two foods have the exact same absorption efficiency, they produce specialists with the same gut characteristics. The foods thus have the same effective richness, but different effective rewards. The larger food also has a larger reward, which gives it a lower $y^*$ and makes it the preferred food of the consumer. Since, these foods produce identical gut characteristics, foods differing only in size can never create coexistence solely based on gut physiology. Depending on the magnitudes of the foods difference in sizes (i.e. differences in effective reward) and their abundances at equilibrium, the generalist consumer may either be behaviorally opportunistic or partially selective.

**Key Predictions**: First, when two foods differ only in their absorption richness, then small differences in richness will be insufficient to produce species coexistence via species-specific differences in gut volume and throughput time.

Second, when the two foods have sufficiently different richnesses as to promote coexistence there will be one species specializing on the rich food, while the other species feeds more opportunistically on both foods 1 and 2. The generalist species will possess a larger gut and a shorter throughput time. (This prediction may have to be adjusted allometrically for body size and metabolic rates.)

Finally, in the special case that foods differ only in size, coexistence cannot be a consequence of species' differences in gut physiology, and in fact foods that differ only in size should produce similar gut physiologies.

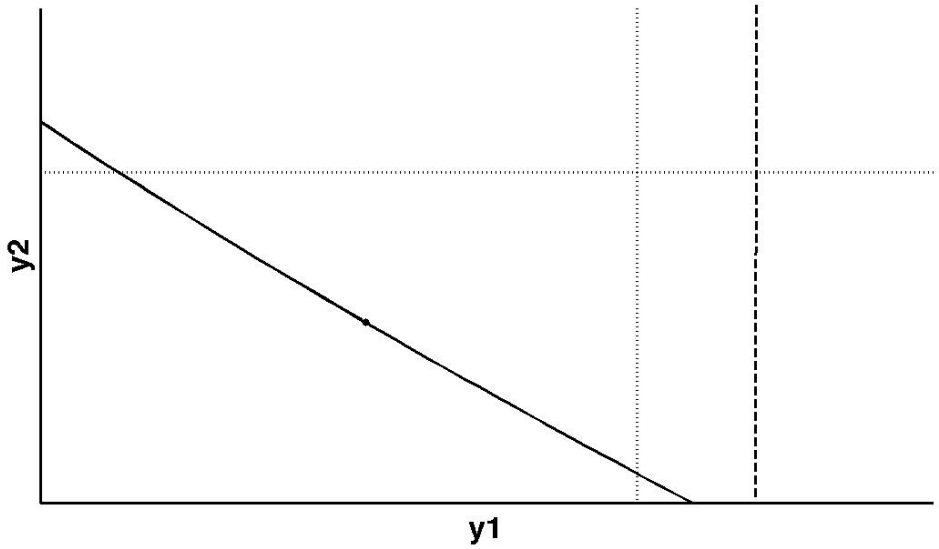


**Figure 4.4:** The effect of coadaptations of foraging behavior and gut physiology on the ZNGIs and behavioral isolegs of consumers feeding on two foods that differ moderately in both absorption rate and bulk. The state space and all lines are the same as in Fig. 1. The dot represents the equilibrium abundances of foods 1 and 2 resulting from consumption by the generalist strategy. Parameters for this figure are: $a_1 = a_2 = 0.1$; $h_1 = h_2 = 1.0$; $\alpha_1 = 1.6$, $\alpha_2 = 0.4$; $e_{max\,1} = e_{max\,2} = 10$; $\chi_1 = \chi_2 = 1$; $b_1 = 1.6$, $b_2 = 0.4$; $c = 0.1$; $\beta = 0.1$; $r_1 = r_2 = 0.001$; $K_1 = K_2 = 100$.

**Qualitative Differences in the Food:** In our model, a purely qualitative niche axis can be achieved in only one way (Table 4.3). It requires two foods that differ in bulk ($b$) and absorption rate ($\alpha$), while holding the ratio of the two constant. We assume that $b_1 > b_2$ and that $\alpha_1 > \alpha_2$. Hence, food 1 is bulkier but is absorbed more quickly, while

food 2 is less bulky but digests more slowly. The specialist on food 1 and the specialist on food 2 both have the same optimal gut size. However, the specialist on food 1 evolves a short throughput time, and the specialist on food 2 evolves a long throughput time. This creates a qualitative niches axis where the specialist on food 1 prefers its food. Its rapid throughput time makes food 2 less rewarding. The specialist on food 2 prefers its food. Food 1 is too bulky and fills its gut.

When the two foods differ only slightly in $b$ and $\alpha$, a compromised generalist strategy will use both resources most efficiently, and this single species is the ESS (Fig. 4.4).

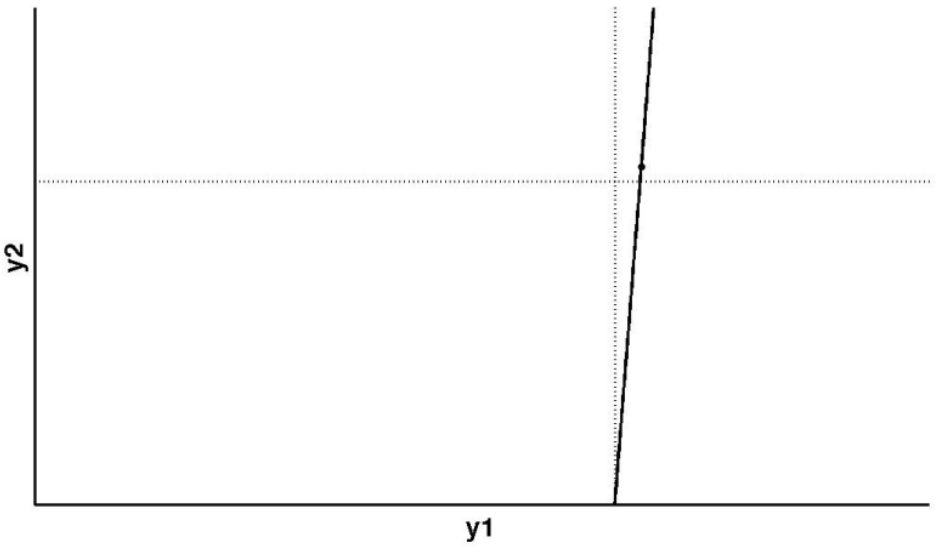


**Figure 4.5:** The effect of coadaptations of foraging behavior and gut physiology on the ZNGIs and behavioral isolegs of consumers feeding on two foods that differ greatly in both absorption rate and bulk. The state space and all lines are the same as in Fig. 1. The Pullliam Isoleg is under the ZNGI of the food 1 specialist strategy and is not visible. The dot represents the equilibrium abundances of foods 1 and 2 resulting from consumption by the generalist strategy. Parameters for this figure are: $a_1 = a_2 = 0.1$; $h_1 = h_2 = 1.0$; $\alpha_1 = 10$, $\alpha_2 = 0$ .01; $e_{max\,1} = e_{max\,2} = 10$; $\chi_1 = \chi_2 = 1$; $b_1 = 10$, $b_2 = .01$; $c = 0.1$; $\beta = 0.1$; $r_1 = r_2 = 0.001$; $K_1 = K_2 = 100$.

As foods diverge in their magnitudes of $\alpha$ and $b$, the generalist's strategy shifts towards that of a specialist on food 1. This is because eating an item of the bulky food 1 with a gut adapted for food 2 (long throughput time) severely decreases overall resource use efficiency by filling the gut. On the other hand, a specialist on food 1 that consumes an item of food 2 can increase its resource use efficiency. In this case, consuming food 2 does not add much to fitness, because food 2 has such a low absorption rate. Because of this, there is a threshold in the differences between $b$ and $\alpha$ beyond which the ZNGI of the opportunistic generalist is right of its Pulliam Isoleg. This means that the generalist cannot be at a behavioral and gut strategy equilibrium with its resources – this point cannot be an ESS. The ESS community will be two specialist species (Fig. 4.5). The two specialists do not have to have exactly the same resource conversion efficiencies, $e_i$'s, (or gut sizes) on the two different foods in order to produce a two-species specialist community, but the efficiencies must be relatively close in value.

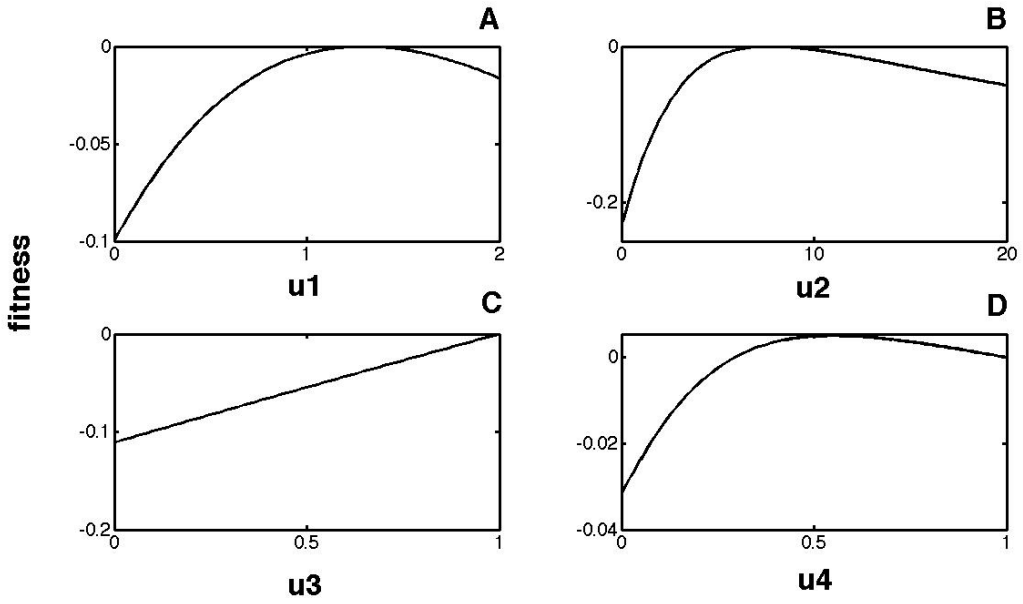


**Figure 4.6:** Adaptive landscapes of the four control variables; $u1$ = gut size, $u2$ = throughput time, $u3$ = probability of accepting food 1, and $u4$ = probability of accepting food 2. These adaptive landscapes correspond to isolegs and ZNGIs of Fig. 1. These landscapes are not evolutionarily stable and selection will drive the landscapes to those depicted in Fig. 4.7.

Figs. 4.6 and 4.7 depict the adaptive landscapes and evolutionary dynamics of generalist and the specialist strategies, respectively. The ecology of these two strategies is depicted in Fig. 4.5. The landscapes in Fig. 4.6 are not evolutionarily stable, as the consumer's fitness is greatly reduced by accepting any food 2. This provides strong selection for behavioral and physiological specialization on food 1. Once the consumer has completely specialized on food 1, the landscapes will correspond to Fig. 4.7.

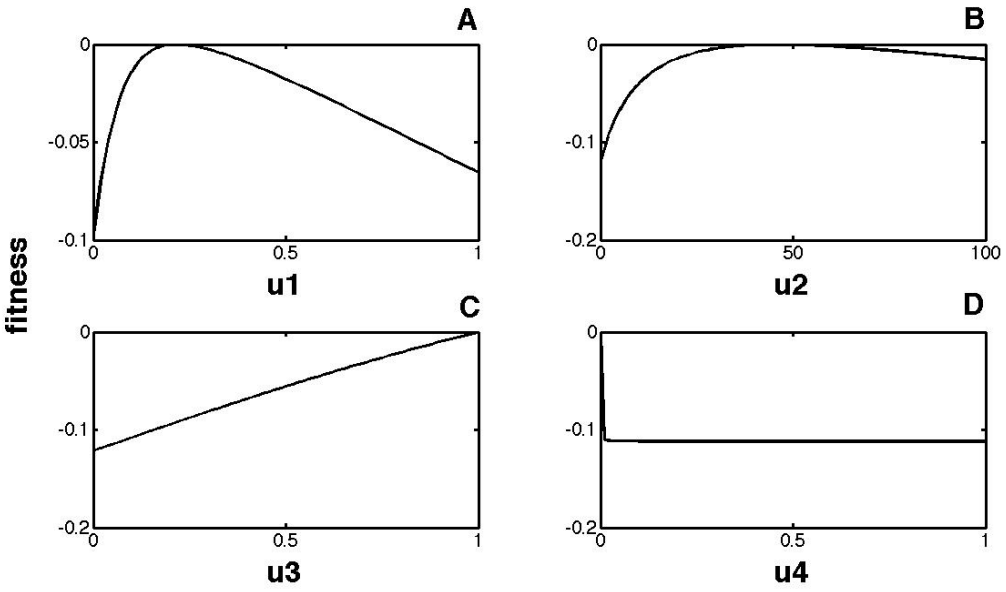


**Figure 4.7:** Adaptive landscapes of the four control variables; $u1$ = gut size, $u2$ = throughput time, $u3$ = probability of accepting food 1, and $u4$ = probability of accepting food 2. These adaptive landscapes correspond to isolegs and ZNGIs of Fig. 1. These landscapes depict the evolution of the specialist on food 1 that comprises one species of the ESS.

**Key Predictions:** First, when two foods diverge along a qualitative niche axis, an ESS of one generalist species arises when foods are relatively similar.

Second, when foods are sufficiently different along the qualitative niche axis, the ESS community will consist of two specialist species, one on each resource. The specialist species will have relatively similar gut sizes. The specialist on the bulkier but easier to digest resource will have a much smaller throughput time than the specialist on the less bulky but harder to digest resource.

In sum, differences in $e_{max}$, $b$, and $\alpha$ will each select for different coadaptations of gut size and throughput time, and represent quantitative niche axes. In contrast, only

differences in the magnitudes of α and *b*, while holding their ratio constant will result in a qualitative niche axis.

### 4.4 Discussion

The resources found within a consumer's environment often vary in properties that influence digestive processing; as such, they provide opportunities for different communities of consumers based on coadaptations of consumer behavior and gut physiology. Our mechanistic model provides a framework for this problem, and predicts the ESS communities resulting from the coadaptation of behavior and physiology to pairs of foods differing in reward (energy/handling time) and richness (energy/bulk).

Different foods select for different specialized gut physiologies. Animals optimize their harvest rate by balancing conversion efficiency, gut fullness, and costs. Our model indicates that large guts signify specialization on poor resources. It can be the optimal strategy in response to bulky resources (low absolute richness), poor absorption rate, or high costs. This agrees with results from physiological studies of gut plasticity where gut size increased in response to poor quality foods in grasshoppers (Yang and Joern 1994) and migratory birds (McWilliams and Karasov 2001). In comparing specialists, most conceivable differences in food properties result in changes in gut size and throughput time. Most of these differences promote a quantitative niche axis. Contrary to this, when two specialists differ only in their throughput times, food qualities are equal, but foods differ in time required for digestion. These qualitative food combinations require specific differences such as simultaneous differences in bulk and absorption rates.

When two food types occur together, they can select for different ESS communities, depending on the differences in the foods. Quantitative differences in foods, if great enough, can lead to a community of one specialist on the richer resource and one partially selective or opportunistic generalist. Once a specialist evolves to feed on the richer resource, then a generalist will evolve to use both resources; it is always optimal to accept the richer resource. Specialists on rich resources are common in nature – carnivores and insectivores. In the presence of these predators, we find opportunistic generalists. For example, periodical cicada emergences provide a pulse of food that is utilized by many animals, including ducks and squirrels (Williams, 1995). In an extreme example, white tailed deer have been known to eat bird eggs from nests (Pietz and Grandfors 2000). Specialists on poor resources, such as grazers, are not predicted to evolve in the presence of richer resources. Including additional coadaptations in the model can explain their evolution. This is discussed further in the following sections.

In our model, a positive covariance between bulk and absorption rate provides the easiest means for creating qualitative differences between foods. Such differences can produce an ESS with two specialist species. More compact but harder to digest foods versus bulkier yet easier to digest foods will create such differences. For this to happen in nature there likely are tradeoffs in the gut relating to gut configurations that directly influence absorption rates. This may require additional gut physiological strategies that we have not included in our model.

In the following, we apply the predictions of our model to the East African ruminant community, then we compare and contrast our modeling approach to those of

others, and finally, we discuss the implications of introducing additional coadaptations into our model.

### 4.4.1 Application to the East African Ruminants

Hofmann (1973, 1989) proposed a classification of ruminants based on their morpho-physiological adaptation to different diet types (high quality browse versus low quality grass and roughage). Hofmann (1989) recognized concentrate selectors (CS), that feed selectively on dicot leaves and utilize plant cell contents (e.g., nonstructural carbohydrates); grazers (GR) that feed on grass and roughage and utilize plant cell wall and fiber; and intermediate types (IM), that switch between feeding as concentrate selectors and grazers, depending upon season. The results of our models appear to fit Hofmann's classification of feeding types based on the coadaptation of behavior and gut structure/function.

According to Hofmann (1989) about 25% of the 150 or so extant ruminant species fall into the GR group, feeding on foods rich in cell wall (structural carbohydrates like cellulose). GR feeders rely on microflora within the reticulo-rumen to provision cellulolytic enzymes that make such "low quality" foods sufficiently rich for subsistence. GR feeders have relatively large reticulo-rumen and long throughput times.

About 40% of the species fall into the CS group. The gut and associated microflora of these species are less capable of digesting cell walls, and instead, CS species select plants rich in soluble (nonstructural) plant cell contents. These nutrients are easier to digest and absorb. The remaining 35% of species fall into the IM group. These species possess guts that are intermediate in structure and function between those of the GR and CS species. IM species forage selectively and avoid fibrous foods when

possible, but they often eat a mixed diet. IM species are capable of adjusting gut structure and function to seasonal changes in forage quality. Like CS species, the guts and microflora of IM species are poor at processing fibrous forage. Both CS and IM species have guts with relatively small reticulo-rumen, fast absorption and short throughput times.

In our analysis, several foods seem to produce guts typical of CS and IM ruminants. These are those foods with intermediate richness, and either high (foods 5, 6, 9, 10) or low (foods 7, 8, 11) absorption rates, α. Foods with high absorption rates possess guts that appear similar to those of CS species, whereas those with low absorption rates may be more similar to IM species. Given the values of the parameters we used in our analyses, we do not see foods that select for both large guts and long throughput times. Additional numerical analysis, however, confirms that such guts do result if we reduce absorption rates to even lower values than we used to generate Table 1 and Fig. 2, while holding richness constant at the intermediate value used in the original analysis.

Only one food (food 14) selected for a gut with large size and short throughput time. This food, with low richness and low α, may represent the gut strategy utilized by African and Asian elephants, which are non-selective browsers. These very large non-ruminants consume foods very high in structural carbohydrates. For their body size, they possess very large guts with short throughput times (Van Soest 1996). Equids (horses) also possess large guts and short throughput times (Boyd and Houpt 1994).

### 4.4.2 Strategies of Modeling Digestive Function

Until the pioneering work of Penry and Jumars (1986, 1987), most models of digestion were compartment models. In these models, the different parts of the GI tract (e.g. stomach, small, and large intestine) represent distinct and homogeneous compartments, each with specific dynamic or static properties. Penry and Jumars (1986, 1987) introduced chemical reactor theory as an alternative paradigm for modeling gut structure and function. They identified the similarity of digestive structures like the reticulo-rumen to that of an industrial batch reactor. Similarly, small intestines appear analogous to plug-flow reactors.

Models based on chemical reactor theory provided a useful conceptual framework for examining digestive processes with respect to gut structure/function and chemical properties of foods. These models assume that all features of the reactor represent a matched set of properties (Karasov 1996). They do not consider the simultaneous changes and adaptations of gut (reactor) traits like size and throughput time. In contrast, the evolutionary game theory model we develop using the fitness-generating approach allows for such adjustments, while the forager simultaneously reaches an ecological equilibrium of consumption and renewal with its resource base.

Our model paradigm demonstrates that in response to some foods (e.g., foods with identical richness but different absorption rates, α's), gut size and throughput times co-vary positively, while in response to other foods (e.g., foods with identical α's but different richness), they co-vary negatively. Another difference in our model from those based on the chemical reaction paradigm is that we combine the rates of digestion (hydrolysis) and the rate of absorption into a single parameter, α. This probably faithfully

reflects situations in which the gut is processing simple sugars that require no digestion prior to absorption. For substrates that require both hydrolysis and absorption, it can be considered a mathematical convenience. These processes are generally incorporated into reactor-based models as separate parameters, and they can be in our modeling approach if deemed necessary.

### 4.4.3 Additional Coadaptations

Our model assumes that the food characteristics of bulk, absorption rate, and maximum conversion efficiency are fixed and equal for all competing consumers. More realistically, these characteristics are properties of both the foods and the consumers themselves. For instance, the evolution and modulation of digestive enzymes can affect both $e_{max}$ and $\alpha$. Starlings, Mimids, and Thrushes all lack the enzyme sucrase. When fed sucrase, these birds suffer from osmotic diarrhea (Malcarney et al 1994). Many birds outside this phylogentic grouping are equipped with sucrase enzymes. Thus, for the Starlings, Mimids, and Thrushes, fruits high in sucrose content potentially represent zero or negative maximum conversion efficiency and zero absorption rate. But, for bird species with sucrase, these fruits represent valuable resources with positive values of $e_{max}$ and $\alpha$.

A consideration of gut chemistry and gut ecology (microflora communities) can provide strong tradeoffs in digestive efficiency for different food types, through the additional coadaptations of maximum conversion efficiency and absorption rate. In our model, most foods differ quantitatively, that is, a single food is richer and becomes the preferred food item in a community of consumers. Tradeoffs in the presence/absence or relative concentrations of digestive enzymes may transform foods that otherwise would

represent a quantitative niche axis into foods that now lie on a qualitative axis. With this consideration, there is the potential that foods differing in their nutritional constituents may create evolutionary minima in adaptive landscapes (Brown and Pavlovic 1992, Abrams and Matsuda 1993). Thus, tradeoffs in gut physiology and their coadaptations with behavior may provide an important mechanism of adaptive speciation (Geritz et al. 1997, Doebeli and Dieckman 2000). Diversification along dietary niche axes is common within many groups of animals.

Prey preparation is another possible coadaptation. Animals frequently modify food items before ingestion. For example, Kaspari (1990) studied Grasshopper Sparrows that often remove the wings and legs of grasshoppers before swallowing them. He showed that prey preparation was an increasing function of both gut fullness and prey abundance. Essentially, prey preparation is a mechanism by which animals decrease the bulk of food at the expense of increased external handling time. In relation to our model, this is expected to evolve when equilibrium abundances of foods are relatively high and consumer guts are relatively full (at equilibrium). Increases in fixed (e.g. predation) and/or variable costs (i.e. gut expensive to maintain) create these conditions. From a consumer's point of view, the coadaptation of prey preparation can change both the richness and rewards of foods. This, in turn, can result in changes of how a consumer ranks resources relative to one another.

### 4.4.4 Conclusions

We contribute a modeling paradigm integrating gut physiology and consumer-resource theory, and we provide testable predictions of how gut physiology and foraging behaviors should co-adapt and contribute to community organization. We predict that foods that

are sufficiently different quantitatively will produce communities of a selective specialist on the richer resource and a generalist that is either partially-selective on the poor resource or opportunistically feeds on both resources. The resource-rich specialists will have smaller guts than the generalists that maintain larger guts to feed on the poor resource. The relative throughput times of these coexisting consumers will depend on the exact nature of the foods. We also predict that foods that are sufficiently different qualitatively will lead to communities of two selective specialist species. Both specialists will have similar gut sizes. The specialist on the bulky resource will have a shorter throughput time. These predictions can be tested with behavioral assays, comparative studies of gut physiology, and studies of community organization. The model also invites extensions via additional traits, such as prey preparation, chemistry or digestion, and gut uptake kinetics.

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# 5. Evolutionary Ecology of HPV: Tradeoffs, Coexistence, and Origins of High-Risk and Low-Risk Types

## 5.1 Introduction

The human papillomavirus (HPV) is the most common sexually transmitted infectious agent in the world. It can cause or facilitate cervical cancer and other epithelial malignancies (zur Hausen 1996). Over 100 HPV types (de Villiers et al. 2004) have been identified and are typically classified as low-risk (LR) or high-risk (HR) based on their proclivity to cause cancer. Most HPV types are low risk and only a few, such as HPV 16 and 18, commonly lead to cancer (Bosch et al. 1995). Distinct molecular differences separate low-risk and high-risk HPV types (Doorbar 2006), and their course of infection within a host and their transmission between hosts varies. Yet, the mechanisms linking the evolutionary dynamics of HPV and the sexual activities of their human hosts are not well understood.

Both HR and LR types of HPV have independently evolved twice in the alpha genus (de Villiers et al. 2004) suggesting convergent evolution within HR and LR forms. This suggests that each type represents a phenotypic strategy that successfully exploits consistent ecological opportunities with human populations.

The classic evolutionary tradeoff for viruses is virulence versus per-contact transmissibility (Galvani 2003). More virulent phenotypes produce more virions increasing the probability of transmission between hosts. But, excessive virion production may either kill the host or trigger a more vigorous immune response. Natural

selection often favors intermediate phenotypes, although some ecological circumstances may promote extremes of persistence or virulence (Frank 1996, Messenger et al. 1996).

No HPV type is particularly virulent in the sense of directly causing host death. Perhaps because HPV is vulnerable to vigorous immune responses, the majority of HPV infections clear within one year (Giuliano et al. 2008). LR types, which produce more virions, are typically cleared by the immune system more quickly than HR types (Insinga et al. 2007). The significance of these differences will become apparent below.

We propose that the central trade-off governing HPV phenotypes is virion production, which affects both per-contact transmissibility and the length of infection prior to immune clearance. We hypothesize that this produces two divergent strategies. LR HPVs use high virion production to maximize transmissibility per contact while tolerating a shorter persistence time due to immune stimulation. HR HPVs produce fewer virions, which reduces the per-contact transmission rate but permits longer infection time and a greater number of sexual contacts.

We find that the relative advantage of each HPV strategy depends on the sexual behaviors within a human population. In our model HR gains an ecological advantage in a host population with sexual behavior characterized by monogamous relationships maintained for months to years. LR gains an ecological advantage in a host population in which individuals are serially monogamous having a high turnover rate of partners. Here, we develop a simple ecological SIR (Susceptible-Infectious-Resistant) model that includes the sexual behaviors of the host population. We demonstrate that sub populations will exert different selection pressure on HPV resulting in speciation into LR and HR phenotypes. In reality, of course, most human populations exhibit great diversity

of sexual activities and individual behaviors change with time. We find this promotes ecological coexistence of HPV types. Finally, we examine the dynamics of removing dominant populations from the HR niche through vaccination and the likely evolutionary consequences.

## 5.2 Model Description

We start with a susceptible-infected-resistant (SIR) model for the epidemiology of HPV in humans (Kermack and McKendrick 1927) (Table 5.1). We let the humans be celibate or in a relationships resulting in 9 different states (Figure 5.1). There are three celibate states of Susceptible (*S*), Infected (*I*), or Resistant (*R*). There are six states representing the possible relationships between *S*, *I*, and *R* individuals: *SS*, *SI*, *SR*, *II*, *IR* and *RR* (Table 1).

**Table 5.1:** Equations governing the dynamics of the model.

| Equation | Description |
|---|---|
| $\frac{dS_{Ci}}{dt} = \mu(N_i - S_{Ci}) + (\delta_i + \mu)\left(S_{Si} + \frac{1}{2}(S_{Ii} + S_{Ri})\right) - (\eta_i + m)S_{Ci} + mS_{Cj}$ | Growth rate of celibate susceptible |
| $\frac{dI_{Ci}}{dt} = (\delta_i + \mu)\left(I_{Ii} + \frac{1}{2}(S_{Ii} + I_{Ri})\right) - (\eta_i + z(u) + \mu + m)I_{Ci} + mI_{Cj}$ | Growth rate of celibate infected |
| $\frac{dR_{Ci}}{dt} = (\delta_i + \mu)\left(R_{Ri} + \frac{1}{2}(S_{Ri} + I_{Ri})\right) - (\eta_i + \mu + m)R_{Ci} + z_i(u)I_{Ci} + mR_{Cj}$ | Growth rate of celibate resistant |
| $\frac{dS_{Si}}{dt} = \eta_i\left(\frac{S_{Ci}^2}{S_{Ci} + I_{Ci} + R_{Ci}}\right) - (2\mu + \delta_i)S_{Si}$ | Growth rate of susceptible in relationship with susceptible |
| $\frac{dS_{Ii}}{dt} = 2\eta_i S_{Ci}\left(\frac{I_{Ci}}{S_{Ci} + I_{Ci} + R_{Ci}}\right) - (2\mu + \delta_i + \alpha_i\beta_i(u) + z_i(u))S_{Ii}$ | Growth rate of susceptible in relationship with infected |
| $\frac{dS_{Ri}}{dt} = 2\eta_i S_{Ci}\left(\frac{R_{Ci}}{S_{Ci} + I_{Ci} + R_{Ci}}\right) - (2\mu + \delta_i)S_{Ri} + z_i(u)S_{Ii}$ | Growth rate of susceptible in relationship with resistant |
| $\frac{dI_{Ii}}{dt} = \eta_i\left(\frac{I_{Ci}^2}{S_{Ci} + I_{Ci} + R_{Ci}}\right) - (2\mu + \delta_i + 2z_i(u))I_{Ii} + \alpha_i\beta_i(u)S_{Ii}$ | Growth rate of infected in relationship with infected |
| $\frac{dI_{Ri}}{dt} = 2\eta_i R_{Ci}\left(\frac{I_{Ci}}{S_{Ci} + I_{Ci} + R_{Ci}}\right) - (2\mu + \delta_i + z_i(u))I_{Ri} + 2z_i(u)I_{Ii}$ | Growth rate of infected in relationship with resistant |
| $\frac{dR_{Ri}}{dt} = \eta_i\left(\frac{R_{Ci}^2}{S_{Ci} + I_{Ci} + R_{Ci}}\right) - (2\mu + \delta_i)R_{Ri} + z_i(u)I_{Ri}$ | Growth rate of resistant in relationship with resistant |

For simplicity, we assume that the total sexually active human population size, $N$, remains constant and gender implicit (allowing it to slowly change does not alter the results). Individuals that die or become permanently non-sexually active are replaced by adding new individuals to the susceptible celibate pool. We let celibate individuals

encounter each other at random, and that upon encounter, individuals have some probability of entering into a relationship ($\eta$). Relationships have a probability of breakup ($\delta$) or a relationship may also end by the "death" of a member. When a relationship ends, surviving members return to their corresponding celibate states.

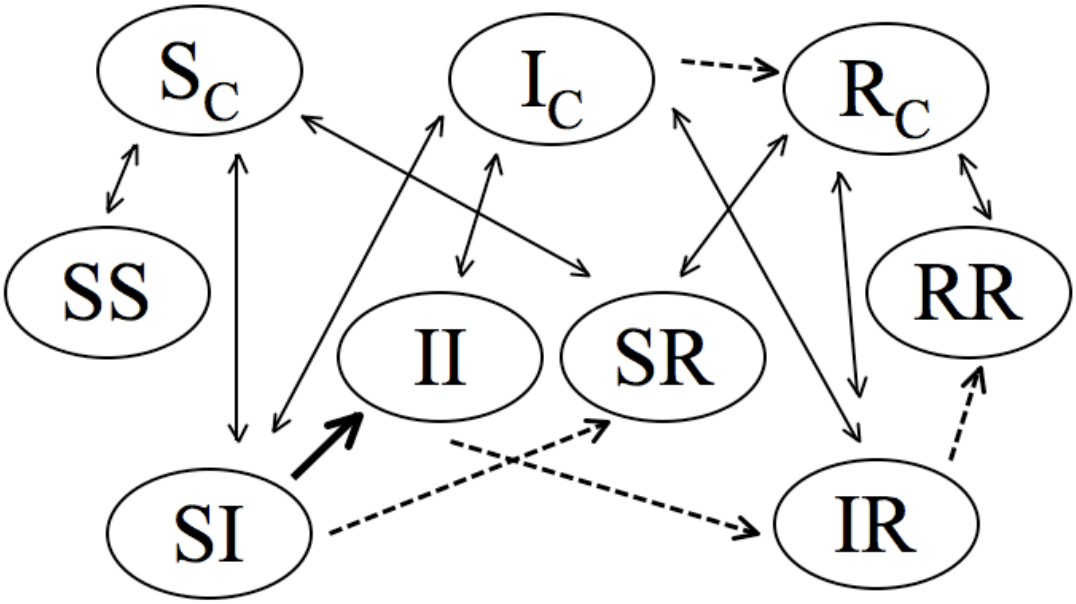


**Figure 5.1:** The social dynamics for how individuals transition from being celibate to being in sexual relationships (thin solid arrows), and the transmission dynamics of Human Papillomavirus (HPV) as it spreads by infecting susceptible (thick solid arrow) and declines as infected individuals become resistant (dotted arrows). The nine states include the three celibate pools of individuals ($S_C$, $I_C$, and $R_C$), and the six combinations of relationships. The challenge for the HPV virus emerges from the limited opportunity for spread. Only one state ($S_I$) provides an opportunity for new infections (with transmission rate $\alpha\beta$), while all four states with infected individuals ($I_C$, $S_I$, $I_I$ and $I_R$) provide opportunities for elimination (with transition rate of $z$). Table 1 gives the equations describing the mathematical model corresponding to the graphical compartmental model depicted here.

HPV infection dynamics are determined by the rate of infection of *S* individuals (*S* becoming *I*), and the rate of infection clearance by *I* individuals (*I* becoming *R*). New infections can only occur within an *SI* relationship. The rate of infection for *S* individuals in a *SI* relationship is determined by the frequency of sex ($\alpha$) and the per sex transmission probability ($\beta$).

We let all *I* individuals, regardless of relationship status, have the same probability ($z$) of clearing the HPV infection. From the virus's perspective, only one of the nine states (*SI*) can augment its prevalence, while any one of 4 states (*I*, *II*, *SI*, and *RI*) can result in a reduction. These positive and negative states represent the main selection forces that drive HPV evolution. We consider two scenarios. The first imagines a population where all individuals conform to a single sexual culture. The second considers two somewhat different sexual subcultures. Sexual subcultures are defined by the values of the parameters ($\eta$ and $\delta$) that describe how frequently individuals enter into and/or break-off relationships. In the two-subculture scenario, for simplicity, we assume that sexual relationships are exclusively within the subculture. We create a mixing of subcultures by having celibates switch between subcultures at some constant per capita rate ($m$). Throughout, we let subculture 1 have a relatively lower rate of relationship turnover (low values for both $\eta$ and $\delta$) and let subculture 2 have a relatively high rate of relationship turnover (high values for $\eta$ and $\delta$).

While the model is couched in terms of the dynamics of the human population, it also describes the "ecology" of an HPV strain. To see how evolution by natural selection acts on the HPV, we let the HPV evolve an evolutionary strategy (heritable phenotypes) representing a tradeoff between transmission ($\beta$) and persistence ($z$). We let $v$ denote the

evolutionary strategy that characterizes a focal HPV infection. We set the transmission probability to $v$: $\beta(v) = v$. We let the clearance rate $z$ be the reciprocal of average persistence time of an infection: $z(v) = 1/\gamma(1-v)$. As the transmission probability ($v$) increases, persistence time of the infection decreases and the clearance rate increases. The variable $u$ is vector-valued and describes the strategies of the HPV strains already present in the population. The strategy of a focal HPV infection ($v$) becomes important in determining fitness and the fate of a rare mutant HPV strains within a population dominated by HPV types using strategies $u = u_1, u_2, \ldots u_n$. The resulting model is an evolutionary game in that the value of an HPV strain possessing strategy $v$ depends upon the HPV strains already present in the population.

## 5.3 Results

### 5.3.1 *Dynamics of HPV prevalence*

When introduced into a population of susceptible individuals, the virus will either die-off or establish. Extinction occurs when rates of having sex, rates of transmission per sex act, probability of relationship break-ups, and probability of entering a relationship are too small; or when the rate of clearing the infection is too high. If the virus is successful, the model dynamics converge on a stable distribution of individuals among the different states (Figure 5.2). The equilibrium prevalence (proportion of population in the infected state) within the population increases with the rate of relationship transitions (both break-ups and unions), and transmission rates (product of rate of sex and transmission per sex act). Equilibrium prevalence declines with the clearance rate (Figure 5.2).

Despite a stable distribution of individuals among the nine states, the actual life experience of any given individual varies. The model can be viewed from an individual-based perspective by "rolling the dice" for an individual to see whether they exit the population of sexually active individuals, enter into, or end a relationship for any given time step of the model. In this way, each individual has a unique life history. The frequency distribution for the lifetime number of sexual partners of individuals within the population (Figure 5.3) varies with subculture (low versus high turnover). Yet, one cannot be certain about an individual's subculture simply based on the experiences of that individual. Even with slow turnovers of relationships, some individuals will still have numerous partners, while even with fast turnovers some individuals will remain celibate or monogamous their entire lifetime. However, of importance to HPV evolution is the average behavior of individuals within each subculture. Natural selection acting on HPV will be driven primarily by average behaviors.

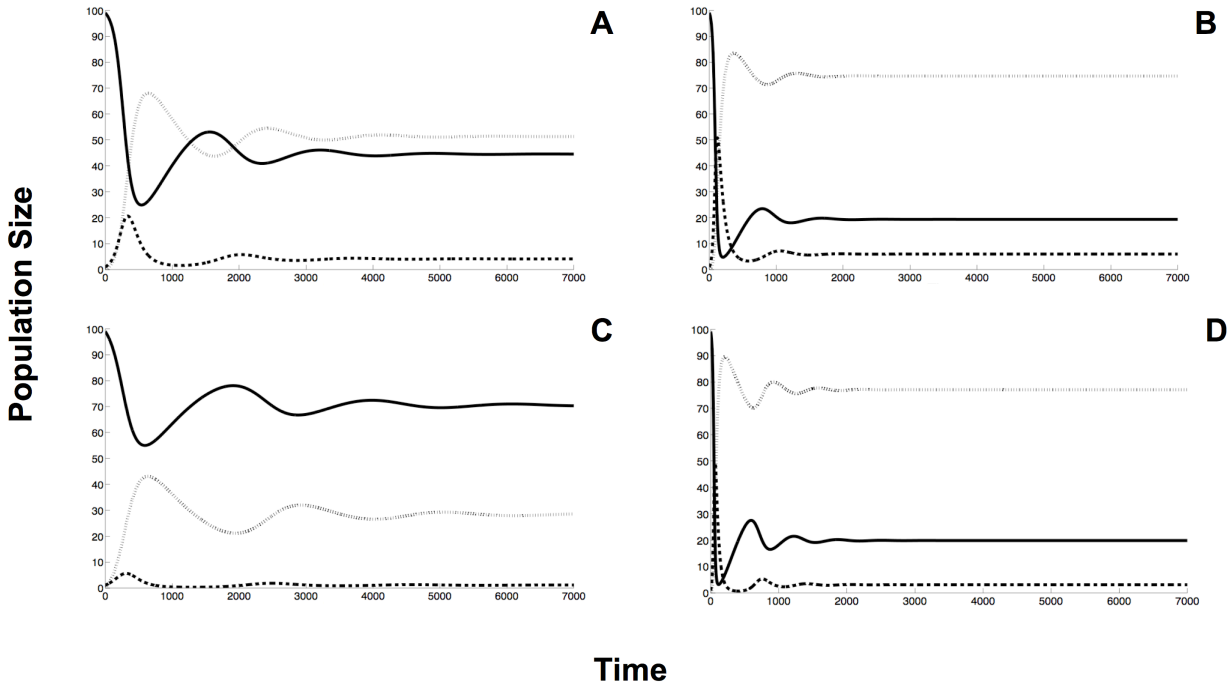


**Figure 5.2:** Infection dynamics for four situations corresponding to A: low turnover culture with high risk HPV, B: high turnover with high risk HPV, C: low turnover culture with low risk HPV, and D: high turnover with low Risk HPV. The graphs show the dynamics of Susceptible (solid lines), Infected (dashed lines) and Resistant (dotted lines) individuals within the population (regardless of whether they are currently celibate or in relationships) following the introduction of HPV into the populations. Parameters common to both subcultures are $\gamma$=100, $\mu$=0.001, and $\alpha$=1. Parameters specific to the high turnover subculture are $\delta$=0.1 and $\eta$=0.1, and for the low turnover subculture are $\delta$=0.5 and $\eta$=0.5. The strategies of the high-risk and low risk are $u$=0.2 and $u$=0.6, respectively.

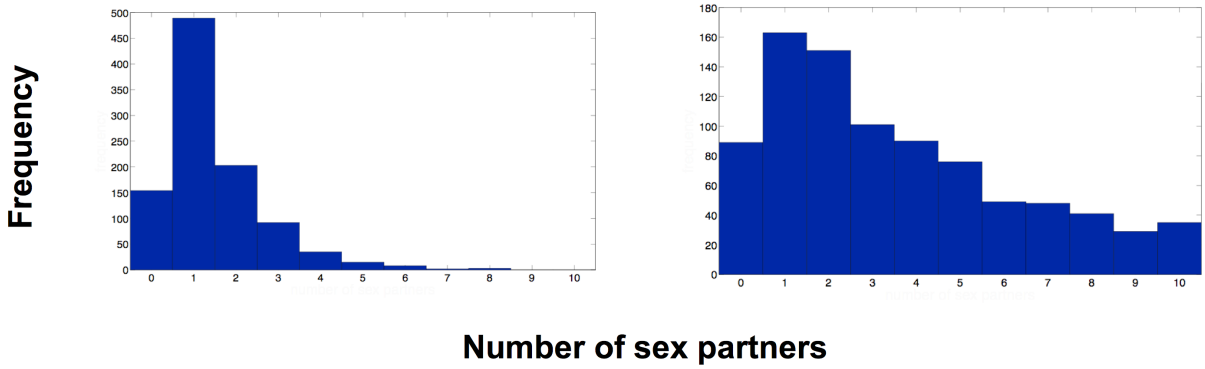


**Figure 5.3:** Histograms of the lifetime number of sex partners for 1000 individuals from a low (left panel) and high (right panel) turnover subculture. Note how both subcultures have the same modal number of partners. With a high turnover of partners the tail of the distribution is stretched to include some individuals having many more than 5 partners. Parameters for this model are $\mu$=0.01 for both subcultures, $\delta$=0.01 and $\eta$=0.05 for the low turnover subculture, and $\delta$=0.1and $\eta$=0.1 for the high turnover subculture.

### ***5.3.2*** *HPV Evolutionary Dynamics within Sexual Subcultures*

In terms of natural selection, phenotypes of HPV that can persist on the lowest equilibrium level of susceptible individuals will replace and outcompete other phenotypes. Of note, only closely related HPV types such as HPV 45 and 18 appear to compete in this manner (Roden et al. 1996). However, competition among closely related variants is sufficient to drive evolution. Under this evolutionary pressure, HPV evolves toward a balance of persistence and transmissibility that minimizes the necessary frequency of susceptibles.

Within this context, phenotypes play a consumer-resource game. This is because the level of resources (susceptible individuals) depends upon the phenotypes present and their abundances. The level of resources in turn determines each phenotype's fitness. We define viral fitness as the per capita growth rate of infected individuals. The fitness of a

phenotype depends on the phenotypes used by other viruses. The solution to such a consumer-resource game is known as an Evolutionarily Stable Strategy (ESS) (Maynard-Smith 1982). An ESS is both unbeatable and convergent stable (Vincent and Brown 2005), meaning that the evolutionary dynamics converge on an optimal strategy.

We begin by analyzing evolution in populations with a single sexual culture ($m$ = 0). We vary the sexual behavior of the population by varying the $\eta$ and $\delta$ parameters. Cultures with higher $\eta$ and $\delta$ have a higher average number of lifetime sexual partners (see Figure 5.3). The virus's ESS level of transmissibility increases with lifetime sexual partners (Figure 5.4). Thus, high turnover populations select for a LR type of HPV and low turnover populations select for HR HPV. The next section shows how different sexual subcultures within the population may explain the speciation and origins of LR and HR HPV types.

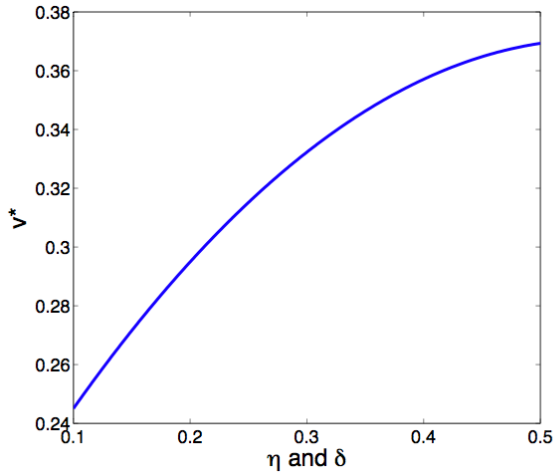


**Figure 5.4:** The ESS of HPV as a function of the relationship parameters. Increasing both $\eta$ and $\delta$ amounts to a higher relationship turnover rate. The y-axis shows the level of transmissibility that will be selected for given the culture's rate of relationship turnover. A high turnover rate selects for a low persistence, high transmissibility HPV (low risk); while a low turnover rate selects for an HPV strain with high persistence but low transmission rate (high risk).

### **5.3.3** *HPV Evolutionary Dynamics with Two Sexual Subcultures*

The model with two subcultures has two sets of equations. Subcultures are linked by the parameter $m$, which determines the per capita rate at which individuals switch from one subculture to the other. What is the effect of the subculture switching rate on HPV coexistence and evolution?

We use adaptive landscapes to display evolutionary dynamics and solve for evolutionary equilibrium. The adaptive landscape plots HPV fitness as a function of a focal HPV infection's strategy for a given ecological circumstance. It shows the ecological performances of individuals from a range of phenotypes under the specific social circumstances. A convergent stable maximum of the landscape is an ESS.

For a population of evolving organisms, the slope of the fitness landscape at the population's mean strategy determines the direction and speed of natural selection (Vincent et al. 1993). We use adaptive landscapes throughout to lead us to ESS solutions. In doing so, we assume that the ecological dynamics of the system occur much quicker than the evolutionary dynamics.

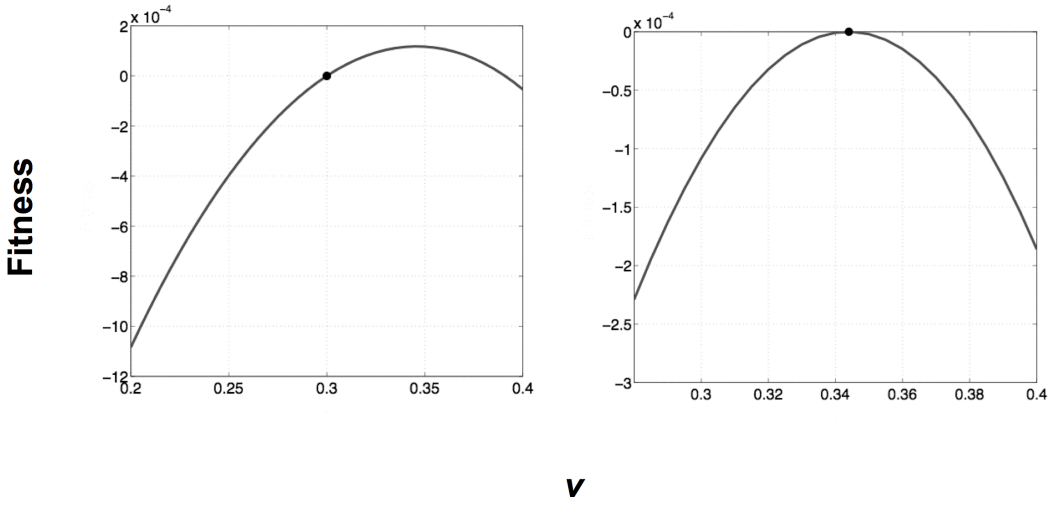


**Figure 5.5:** Adaptive landscapes for an HPV strain facing two subcultures with a high rate of switching of human individuals from one subculture to the other. This situation favors a single generalist HPV strain of $u$=0.345 (right panel), that persists well in both subcultures. The left panel shows the mean HPV strategy at $u$=0.3 left of the ESS. Parameters used for this example are $\gamma$=100, $c$=1, $m$=0.1, $\mu$=0.001, $\delta_1$=0.05, $\delta_2$=0.5, $\eta_1$=0.2, $\eta_2$=0.5, $\alpha$=1.

With rapid switching between subcultures (high values of $m$), most individuals will experience both sexual cultures. From the HPV perspective, this blurs the distinction between them. To the HPV, the two subcultures are a single resource. This creates

selection pressures for the virus to adopt a strategy, that does relatively well in both subcultures. So evolutionarily, relatively high switching rates between subcultures leads to a single HPV with a generalist strategy which is in between the two specialist strains that would be favored for each subculture alone (Figure 5.5). This would be an intermediate phenotype with moderate proliferation and persistence. This would likely be manifest as an HPV type with intermediate cancer risk.

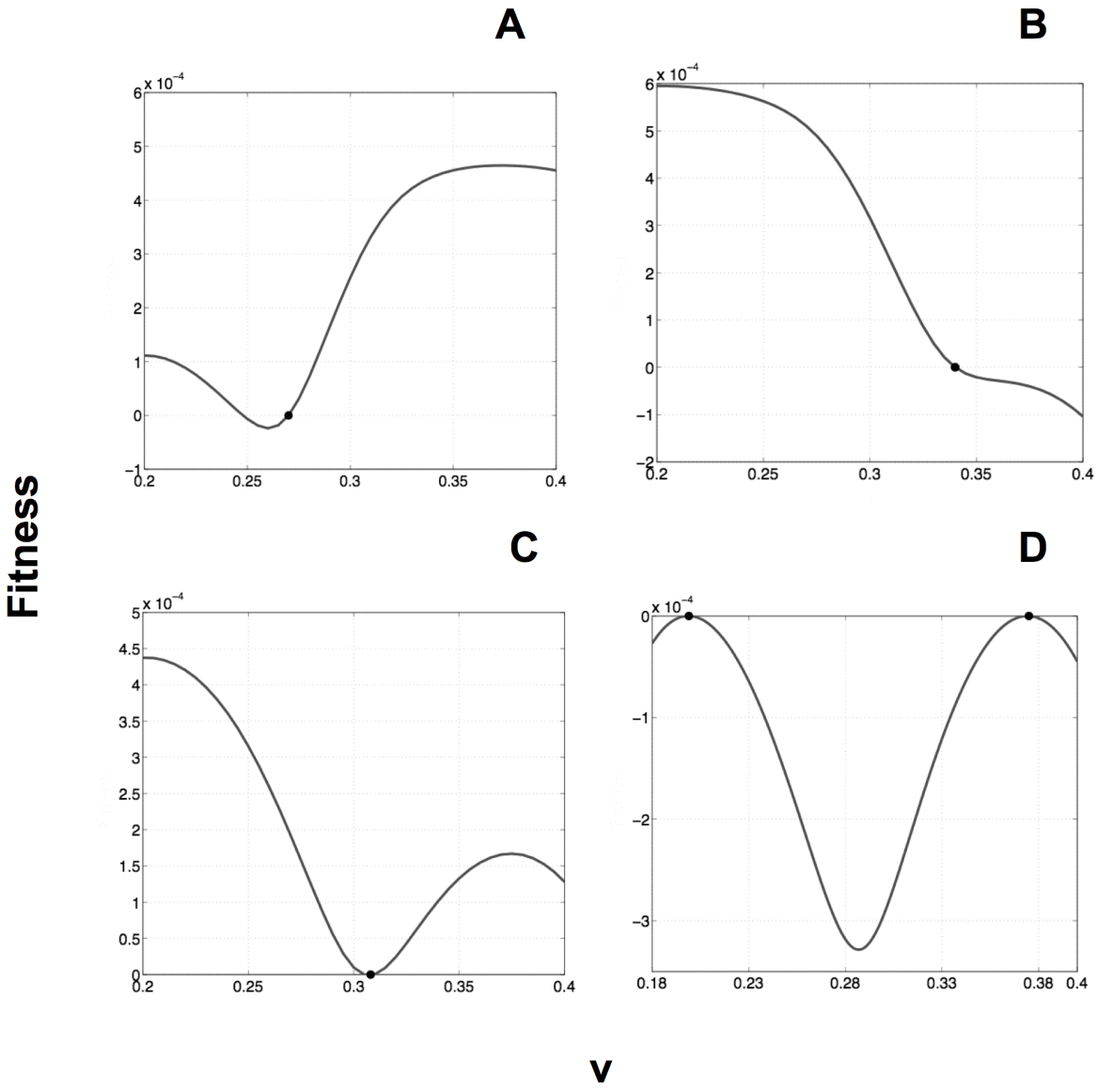


**Figure 5.6:** Adaptive landscapes depicting a situation where the rate of switching by humans between subcultures is relatively low. In this case, the ESS for HPV consists of two strains that specialize somewhat on their respective subcultures. Starting with just a single HPV strain of either $u$=0.27 (6A) or $u$=0.34 (6B) results in a configuration of the adaptive landscapes that results in evolution towards a minimum of $u$=0.308 (6C). This favors speciation of the HPV into two strains. Natural selection can then achieve an ESS where strains $u_1$=0.2 (high risk HPV) and $u_2$=0.375 (low risk HPV) coexist at peaks of the adaptive landscape (6D). Parameters for this example are $\gamma$=100; $c$=1; $m$=0.0005; $\mu$=0.001, $\delta_1$=0.05, $\delta_2$=0.5, $\eta_1$=0.2, $\eta_2$=0.5, $\alpha$=1.

Importantly, a single HPV strain that adopts a generalist strategy is at an evolutionary minimum (Figure 5.6). This minimum is convergent stable (Brown and Pavlovic 1992, Abrams et al. 1993). A strategy sitting at an evolutionary minimum experiences disruptive selection. This promotes adaptive speciation (Geritz et al. 1998, Cohent et al. 1999) so that two resident strains of HPV possessing slightly different strategies can coexist on opposite sides of the minimum. These daughter strains will then diverge and evolve in opposite directions. Thus, the diversity and temporal variation of sexual behavior in individuals within a human population results in an ESS community (epidemiology) of multiple coexisting HPV strains. Many strains possess strategies that are closer to each other than their respective subcultural specialists, because each strategy is now exposed to both subcultures. This explains the large number of HPV types found with human populations throughout the world.

Finally, we note that eradication of a single HPV type from the ESS community of several coexisting types leaves an empty niche. For instance, eradication of the HR type causes the remaining LR strain to evolve to the generalist strategy at the convergent stable minimum (Figure 5.6C). This may lead to speciation and re-establishment of the HR strain. Successful eradication of HR strains may not be evolutionarily persistent as the remaining LR strains evolve to fill the empty niche. However, as genetic studies show (Bernard 2007), the rate of evolutionary change may be too slow to be of importance to current human health.

**5.4 Discussion**

While the majority of HPV studies focus on its molecular biology and clinical outcomes, we view HPV in the context of its evolutionary ecology. This article addresses the following questions: 1.) What evolutionary selection pressures and viral adaptations/strategies result in the observed high and low risk HPV types? 2.) How does variation in human sexual activity contribute to the evolution and persistence of high and low risk types within populations? 3.) What will be the ecological and evolutionary consequences of an HPV vaccine?

Based on mathematical models and computer simulations, we investigate how a tradeoff between persistence and transmissibility coupled with sexual behavior of hosts creates the fundamental selection pressure driving HPV evolution. Fitness for a virus is its ability to spread to susceptible hosts from infected hosts. In our model, HR HPVs uses a stealth approach in which the virus delays the immune response by remaining within the epithelial cells and producing relatively few virions. This phenotype persists for longer periods of time because of the limited immune response (this persistence may predispose these types to transform human cells). Daud et al. (2010) showed that high-risk HPV interferes with the toll-like receptors of the innate immune system as part of its strategy for persistence. However, the penalty of this strategy is the production of fewer virions and reduced probability of transmission at each sexual contact. Clinically, these infections are inconspicuous such as the flat lesions of HPV 16 and 18. On the other hand, the LR, by producing large numbers of virions, achieves higher transmission probabilities per sexual contact. However, this strategy also stimulates the immune system resulting in more rapid elimination. Clinically, this infection manifests as genital

warts (Types 6 and 11), which act as virus "factories." Studies have shown that HR HPVs are more persistent than LR HPVs (Insinga et al. 2007, Koshiol et al. 2010, Louvanto et al. 2010). In terms of transmissibility, Oriel (1971) showed that genital warts result in 60% viral transmission between partners. Of course the more relevant metric is the per sex act transmission, which requires detailed knowledge of sexual contacts and infections status. There is no study that we are aware of to date that has published this information.

For HPV, the value of a given phenotype along this persistence-transmissibility continuum depends on the sexual behavior of the host population, specifically the turnover rate of sexual relationships. To persist, HPV requires some turnover of sexual partners within the host population - exclusive, life-long monogamous relationships do not support HPV. In our model, we consider two sexual subcultures: 1. "low turnover" in which sexual relationships are exclusive and transient but relatively stable lasting, for example, from months to years. 2. "high turnover" in which sexual contacts are more frequent and relationships have a typical duration of days to months. Our models demonstrate that low turnover selects for HR HPV types because longer (but not life-long) monogamous relationships favor a viral strain that has high persistence, but low transmissibility. On the other hand, high turnover favors the LR HPV strategy with high transmissibility but low persistence. We also find that different sexual subcultures within the human population promotes the coexistence of HR and LR HPV types and, under some conditions, can result in selection for intermediate, generalist strains of HPV. Adaptive speciation of these generalists into HR and LR specialization can also occur – consistent with data that both types have independently evolved twice in the alpha genus.

In reality, sexual behavior in humans is highly varied and can change over time under the influence of social factors. Identifying clearly defined groups within the human population is difficult and potentially controversial. Sex workers clearly represent a high turnover group. Ecologically, HR HPVs, particularly HPV 16 are almost invariably more abundant within all studied groups including sex workers (Ishi et al. 2000, Juarez-Figueroa et al. 2001, Choi et al. 2003, Mak et al. 2004). Our model provides several reasons for this observation. HR HPV is more persistent which can lead to higher prevalence than LR. Furthermore, surveys of human sexual activities indicate typical behaviors more consistent with our low turnover subculture (Herbenick et al. 2010, Reece et al. 2010). Seroprevalence studies should indicate more exposure and clearance of LR types if our hypothesis is correct, but a skew in prevalence may make exposure to HR strains more likely. Comparative studies using the same methods are needed – for instance, a comparison of sex workers to low turnover groups within the same population.

Studies of PV in other animals with less varied sexual behavior may be instructive. For example, Bonobos (*Pan paniscus*), use sex for social purposes beyond reproduction such that individuals have frequent sex with multiple partners. Within Bonobos, PV diversity is considerably lower that that found in human populations, consisting of a type evolutionarily related to LR types 6 and 11 (Van Ranst et al. 1992). Thus, in a species with a high-turnover of sex partners, only LR HPV types are present, as our model would predict.

Our model requires several caveats. For the purposes of modeling the factors favoring HR and LR strains, we assumed that all HPV phenotypes compete for

susceptible individuals. Epidemiologically, this amounts to full immune cross reactivity between HPV phenotypes. In reality, this may only be true for very closely related HPV types. There is no cross reactivity between distantly related HR and LR HPV types (Roden et al. 1996). Cross-reactivity itself may be another aspect of HPV's phenotype that promotes the coexistence and diversification of HPV strains. Competition for susceptible hosts produces the divergent selection that explains the origins and evolutionary maintenance of HR and LR HPV types. As the HPV types diverge they may also be under selection to lose cross-reactivity. This results in the "ghost of competition past" (Rosenzweig 1981) in which two previously competing species evolve phenotypes that no longer directly influence each other's fitness or ecology.

We also assume that HPV evolves and responds to natural selection. Molecular clock studies suggest that HPV evolves very slowly – estimated at a rate of 1% nucleotide change per 100,000 to 1,000,000 years (Bernard 2007). Molecular clock studies generally assume a constant rate of evolutionary change. In our model, as the HPV strains traverse the adaptive landscape the rate of adaptive evolution can initially be very high but then slows to zero as the strain approaches its ESS. Thus HPVs may be capable of rapid evolution but appear relatively static when close to their ESS.

Does our model of HPV evolutionary ecology provide insight into management strategies for HPV? There will undoubtedly be an ecological and evolutionary response to the vaccine. Ecologically, it should reduce the prevalence of these types by reducing the resource pool available to them (number of susceptible individuals).

Evolutionarily, the eradication of HR HPV type will leave an open niche, causing other types to evolve and fill this HR niche. This scenario of course relies on the fact that

HPVs compete with one another and are able to evolve sufficiently fast. Perhaps, more realistically, the vaccine will not completely eradicate a targeted HPV type, but rather act as a strong selective force. There has been discussion in the literature about the possibility of these reductions changing the prevalence of other types via interactions between different HPVs (Hughes et al. 2002, Elbasha and Galvani 2005). There is some evidence for associations between specific HPV types (Mejlhede et al. 2010).

Nevertheless, based on our models, absence of an HPV type should select for initial evolution towards a generalist phenotype but there is reasonable probability of eventual speciation back into the original strains. We advise close, long-term monitoring of HPV types within human populations following vaccination efforts to detect evolutionary changes.

In conclusion, an evolutionary ecology perspective of HPV identifies the major selective forces and evolutionary tradeoffs that govern the interactions of HPV with humans. Our approach provides insight into the critical parameters governing HPV-human ecology and identifies key parameters that should be measured in future research.

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# 6. The ecology of HPV lesions and the role of somatic evolution in their progression

## 6.1 Introduction

HPV is causally linked to 5% of human cancers worldwide (Parkin 2006). The molecular reasons for why HPV causes cancer are well sorted out (Doorbar 2006). High-risk HPV E6 and E7 proteins bind and subsequently lead to the degradation of p53 and pRb respectively. The degradation of these proteins causes both chromosomal instability (Duensing et al. 2004) and increased cell proliferation (Hamid et al. 2009). During the progression of HPV lesions, HR HPV E6 and E7 genes are expressed and often integrated into the host cells' chromosomes (Hudelist et al. 2004). Despite the fact that cancer biologists accept the central role of evolution in carcinogenesis, conceptual models describing the events of HPV-caused lesion progression are not framed in an explicit evolutionary context (e.g. zur Hausen 2000, Doorbar 2006, Snijders et al. 2006). In this article, we use mathematical models of HPV lesions to explore the ecology of HPV lesions and the role of evolution in lesion progression.

In the study of HPV-caused lesion progression, two distinct roles of HPV's early proteins are apparent. First, the effects of E6 and E7 (and possibly E5) allow HR HPV to act as a mutagen (Duensing et al. 2004). Second, these same proteins are responsible for the ecological success of HPV infected cells (i.e. tumor forming properties) and are subsequently co-opted by host cells presumably for the same reason – ecological advantage. To be clear, at some point during HPV infection, viral control over a cell can be lost and this cell can become autonomous, resisting both viral and homeostatic tissue controls. We refer to this as initiation. HR HPV proteins appear to be causal in the

development of autonomous cells and their subsequent ecological and evolutionary success.

The functions of HPV's early proteins have clear ecological effects, bearing directly on cellular population dynamics. This is not surprising in light of the fact that HPV causes benign hyper-proliferative skin lesions (i.e. warts). HPV's E5 protein increases the abundance of epidermal growth factor receptors on the cell surface (Straight et al. 1993). HPV E6 and E7 are thought to increase cell proliferation by allowing the cells to continue to divide in the presence of signals to stop (Hamid et al. 2009). Furthermore, E5 and E6 can increase cell motility (Kivi et al. 2008). Cell motility influences population dynamics in this system, since cell migration drives tissue turnover. Cell migration is a double edged sword for HPV, since it is equivalent to cell death as these cells move out of the proliferative compartment, but also allows HPV to continuously shed infectious virions from the skin surface. We use a model of HPV infection in skin to explore the relationship between HPV's early proteins and basic ecological parameters affecting cell population dynamics.

Ecological advantages inherited by cells from HPV are not the only drivers of lesion progression. In fact, LR HPV types appear to have stronger effects on cell population dynamics than the HR types. LR types tend to produce warty infections, whereas HR tends to produce clinically inapparent flat warts (Lowhagen et al. 1993). Arguably, a critical difference between LR and HR HPV is the mutagenic effect of HR HPV. Southern et al. (1997) demonstrated rampant basal cell tetrasomy in benign HR caused lesions. Similar lesions caused by LR types showed no sign of tetrasomy. In an

evolutionary context, the mutagenic effect of HR HPV can potentially initiate a population of autonomous and evolving cells.

We use a model of HPV lesions to explore the hypothesis that initiation of an autonomous cell population occurs in low-grade lesions, and subsequent tissue changes associated with high-grade lesions is the result of adaptive evolution of these autonomous cells. Our model predicts tissue changes qualitatively similar to those seen during lesion progression, by a novel and previously unexplored mechanism.

## 6.2 Model Description

We use two related mathematical models of HPV infection in skin to explore the ecology and evolution of HPV lesions. The first model is a 3-dimensional system of partial differential equations modeling HPV infection in a small cube of skin ($300\mu m^3$). We use this model to explore the relationship between HPV infection and cellular population dynamics. The second model is an extension of the first model. In the second model, we add an evolving population of cells with a phenotypic dimension ($w$). We use this second model to explore the role of evolution in lesion progression. We borrow from the literature to roughly parameterize the models for mucosal tissue (Table 6.1).

**Table 6.1:** Parameter meanings and values used in simulations.

| Parameter | Meaning | Values used in simulations (units) [ref] |
|---|---|---|
| $r_i$ | Maximum per cell growth rate | 0.3 ($day^{-1}$) [Larsson et al. 2008]<br>same for HPV infected cells unless otherwise specified. |
| $K_i$ | Maximum tissue density at which cells can divide | $1x10^5$ (cells $mm^{-3}$) [Lyng et al. 2000]<br>$1.5x10^5$ for HPV infected cells unless otherwise specified. |
| $\alpha_i$ | Cell migration rate | 0.015 (mm $day^{-1}$) [Squier and Kremer 2001, Kang et al. 2011]<br>same for HPv infected cells unless otherwise specified. |
| $\beta$ | Cell diffusion coefficient | $1x10^{-4}$ ($mm^2$ $day^{-1}$) [Tjia and Moghe 2002] |
| $z_{i0}$ | Distance from the basement membrane where 50% of cells differentiate | 0.03 (mm) – uninfected cells<br>0.15 (mm) – HPV infected cells |
| $\eta$ | Sensitivity of differentiation to cell spatial position | 10 ($mm^{-1}$) |
| $\gamma_{max}$ | Maximum virion production rate | 2500 (virions $cell^{-1}$ $day^{-1}$) [Tsai et al. 1996] |
| $z_u$ | Thickness of epithelium | 0.3 (mm) [Kang et al. 2011] |
| $\delta$* | Probability of mutation per cell division | 0.6 (unitless) |

* parameter specific to model 2.

The first model describes the dynamics of five state variables: uninfected proliferating cell density, infected proliferating cell density, uninfected differentiating cell density, infected differentiating cell density, and virion density (Table 6.2). In epithelial tissue, the basement membrane separates keratinocytes from the underlying mesenchyme. We model basal cells, which are attached to the basement membrane using a fixed boundary condition. We assume that the basal cells divide to maintain their density and provide cells, which migrate to the skin surface. As cells move off the basement membrane, they move into a proliferating compartment, representative of

transit amplifying cells. We assume that proliferating cells grow via a logistic term ($T$ is the sum of all cell densities at any point in space). As cells migrate further from the basement membrane, they eventually lose their ability to proliferate and start to differentiate. HPV infection delays cell differentiation longer than in uninfected cells, which differentiate shortly after they leave the basement membrane (Peh et al. 2002). The per capita rate of differentiation is modeled using a beta function (equation 1), and we assume that differentiation is a function of distance from the basement membrane. HPV infected differentiating cells produce virions. In this sense, "differentiation" for infected cells is equivalent to late gene expression. We also assume that virion production is a declining function of distance from the basement membrane (equation 2). Cells migrate to the skin surface at a constant rate $\alpha$. Finally, cells move randomly in space, the rate of movement proportional to the diffusion coefficient $\beta$.

In analyzing the dynamics of HPV lesions, we calculate virion flux (the number of virions shed from the skin surface per day, equation 3) and the total number of HPV infected cells in the lesion (equation 4). We use virion flux as a surrogate for infectivity and viral fitness, to measure the benefit to HPV from modulating specific parameters.

We use Dirichlet boundary conditions for all boundaries. The boundary at the skin surface is set to zero for all variables. For the uninfected cells, the lateral boundaries are set equal to $K_c$, which represents normal homeostatic tissue density. The infected cells and virions are assumed to have zero density at the lateral boundaries of the skin cube. We consider only micro-lesions, which start at the basement membrane in the center of the cube and do not expand to the lateral boundaries. The lower $z$ boundary

representing the basement membrane is assumed to have a constant density of cells equal to $K_c$, regardless of cell infection status.

Initial conditions consist of uninfected cells at homeostatic equilibrium. This means the whole tissue has a constant density of cells equal to $K_c$. To seed the HPV infection, a cluster of cells is initially infected by HPV.

**Table 6.2:** The variables and equations of the models.

| State Variable | Equation governing dynamics |
|---|---|
| Uninfected proliferating cell density | $\frac{\partial c_p}{\partial t} = r_c\left(1-\frac{T}{K_c}\right)c_p - f_c(z)c_p - \alpha_h\frac{\partial c_p}{\partial z} + \beta\nabla^2 c_p$ |
| Infected proliferating cell density | $\frac{\partial h_p}{\partial t} = r_h\left(1-\frac{T}{K_h}\right)h_p - f_h(z)h_p - \alpha_h\frac{\partial h_p}{\partial z} + \beta\nabla^2 h_p$ |
| Uninfected differentiating cell density | $\frac{\partial c_d}{\partial t} = f_c(z)c_p - \alpha_h\frac{\partial c_d}{\partial z} + \beta\nabla^2 c_d$ |
| Infected differentiating cell density | $\frac{\partial h_d}{\partial t} = f_h(z)h_p - \alpha_h\frac{\partial h_d}{\partial z} + \beta\nabla^2 h_d$ |
| Virion density | $\frac{\partial v}{\partial t} = \gamma(z)h_d - \alpha_h\frac{\partial v}{\partial z} + \beta\nabla^2 v$ |
| Evolving cell density* | $\frac{\partial e}{\partial t} = r_e\left(1-\frac{T}{K_e(w)}\right)e + M(\cdot) - \alpha_e\frac{\partial e}{\partial z} + \beta\frac{\partial^2 e}{\partial z^2}$ |

*variable specific to model 2.

$$f_i(z) = \frac{\exp(\eta(z - z_{i0}))}{1 + \exp(\eta(z - z_{i0}))} \tag{1}$$

$$\gamma(z) = \gamma_{\max}\left(1 - \frac{z}{z_u}\right) \tag{2}$$

$$\text{Virion flux} = \alpha_h \int_0^{y_u}\int_0^{x_u} v(t,x,y,z_s)\,dx\,dy \tag{3}$$

$$\text{Number of HPV infected cells} = \int_0^{z_u}\int_0^{y_u}\int_0^{x_u} h_j(t,x,y,z)\,dx\,dy\,dz \tag{4}$$

The second model is an extension of the first model. It contains the same state variables, plus an additional variable, which models an evolving population of autonomous cells (last row of Table 6.1). For ease of numerical solution, we include only 1 spatial dimension (vertical distance from the basement membrane to the skin surface) in the second model. The evolving cells have a phenotypic dimension (*w*). We assume that the cells only evolve their carrying capacity, which is a measure of both maximum density and ecological competitiveness. We also assume that the evolving cells do not differentiate. We borrow from Cohen's (2009) theory of evolutionary distributions to model evolution in a partial differential equation framework. The mutation term *M* (see appendix for specifics), describes the "movement" of cells in phenotype space.

The boundary conditions for the second model are the same as those described for the first model for the variables shared between the models. For the phenotypic dimension *w*, the boundaries represent phenotypic states just below the minimum and above the maximum attainable phenotypes. The density of evolving cells at these boundaries is set to zero.

The second model explores the progression of lesions through evolutionary change. Therefore, initial conditions consist of a steady state HPV lesion. We assume that within a low-grade lesion, an initiation event creates a small population of autonomous evolving cells just above the basement membrane.

We employ the method of lines (Schiesser and Griffiths 2009) to obtain numerical solutions for both models.

## 6.3 Results

### 6.3.1 *Ecological Dynamics of HPV Lesions*

We first use model 1 to first investigate the effect of initial cell type infected on lesion dynamics. Basal cells are attached to the basement membrane, whereas the adjacent suprabasal cells actively migrate to the skin surface. Figure 6.1 shows the difference in the dynamics of HPV infected cells and virion flux when basal cells versus suprabasal cells are initially infected. If basal cells are initially infected, the lesion reaches steady state by roughly 40 days. However, if suprabasal cells are initially infected, the infection is ephemeral and cleared from the tissue by 40 days. In either case, equilibrium is reached by roughly the time it takes for the tissue to turnover twice.

Figure 6.2 shows a 3-dimensional view of the densities of HPV infected cells and virions in a steady state HPV lesion. At steady state, HPV infected cells form a tumor above the cluster of initially infected basal cells by dividing and increasing the tissue density as they migrate up through the skin layers. HPV virions are restricted to the upper layers of the tissue, as infected cells do not produce virions in the lower layers.

HPV causes benign skin tumors. In doing so, HPV must modulate the population

dynamics of the infected cells. We use virion flux as a surrogate for infectivity and viral fitness. We explore the effect of modulating basic ecological parameters on virion flux in steady state lesions. Figure 6.3 shows the effects of infected cell carrying capacity ($K_h$), infected cell intrinsic growth rate ($r_h$), and infected cell migration rate ($\alpha_h$) on virion flux. Increasing infected cell carrying capacity significantly increases virion flux by itself. Whereas, increasing intrinsic growth rate has no effect by itself and decreasing cell migration rate has a comparatively minimal positive effect on virion flux. However, both intrinsic growth rate and migration rate strongly interact with cell carrying capacity. Doubling intrinsic growth rate nearly doubles the effect of increasing carrying capacity on virion flux. And halving cell migration rate more than doubles the effect of increasing carrying capacity on virion flux.

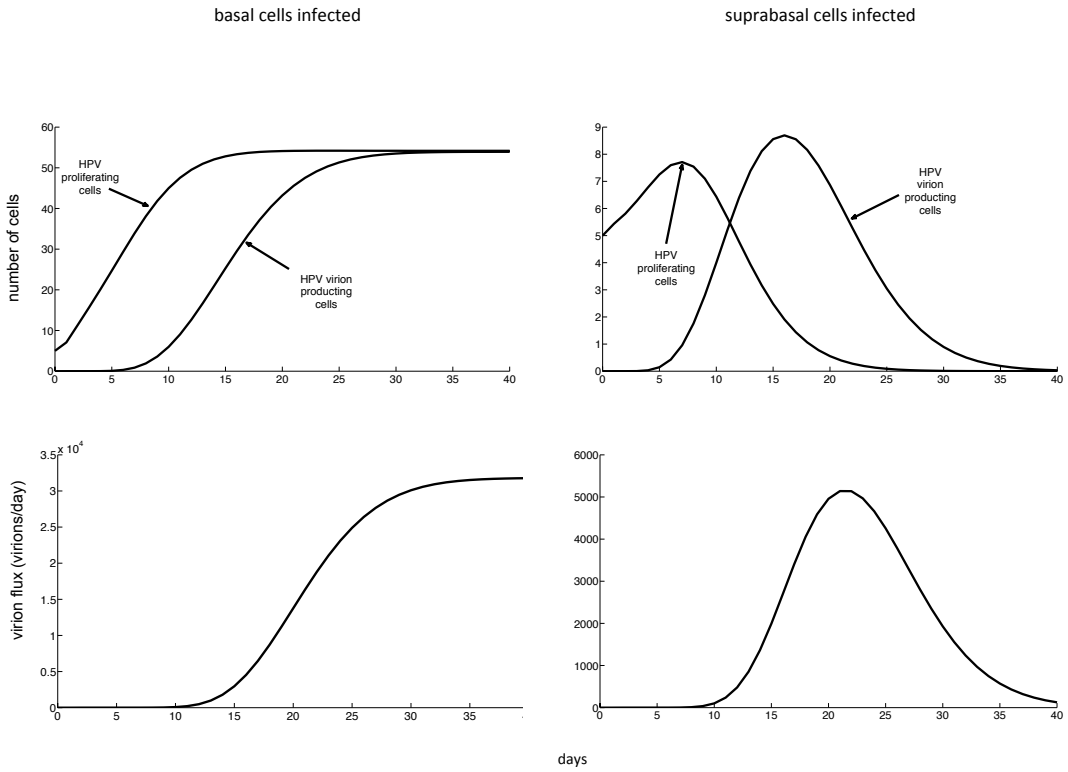


**Figure 6.1:** Cell and virion flux dynamics in HPV lesions. The left and right panels show the dynamics of lesions when basal cells and suprabasal cells, respectively are initially infected.

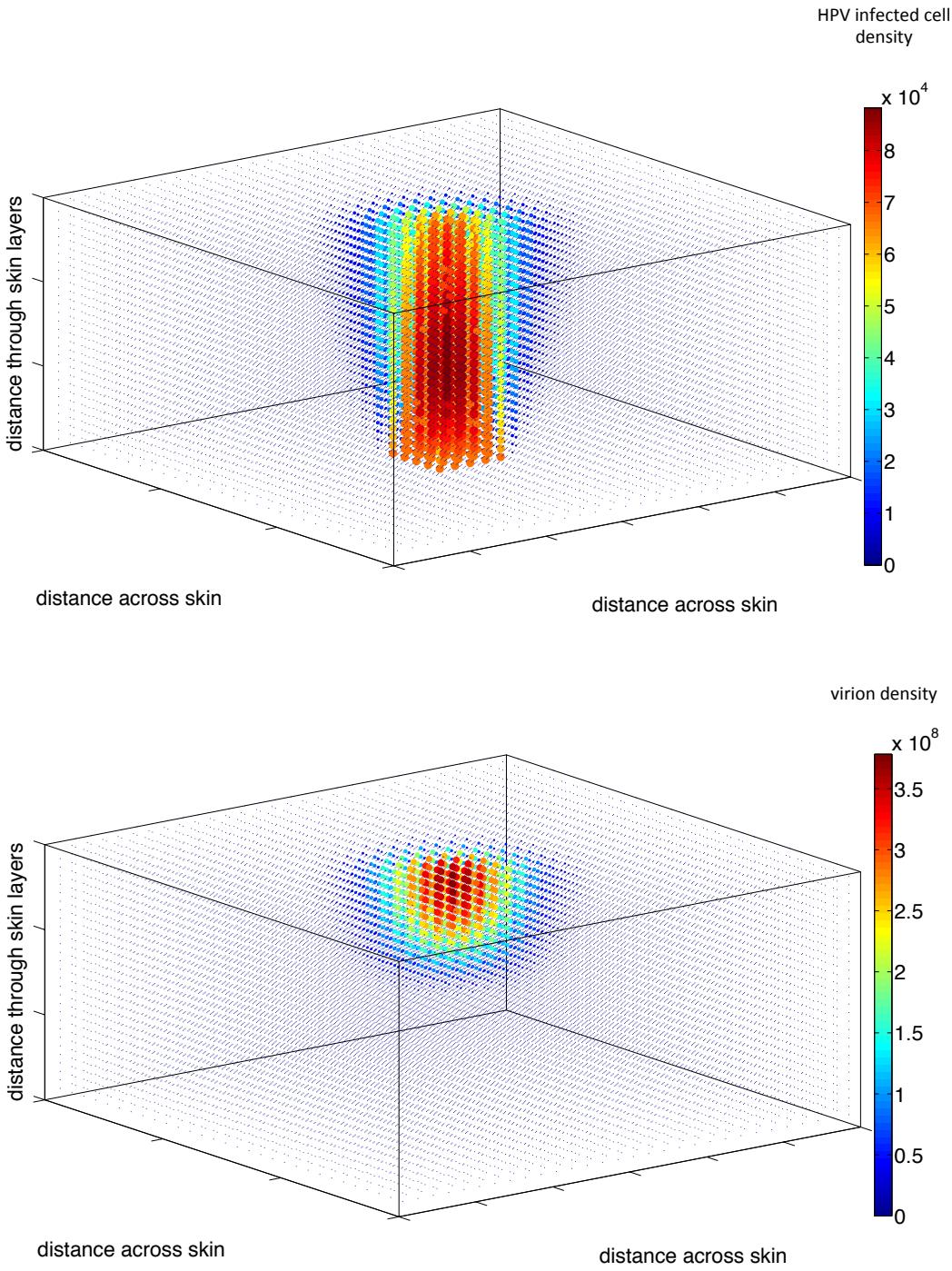


**Figure 6.2:** The density of HPV infected cells and virions in a steady state lesion.

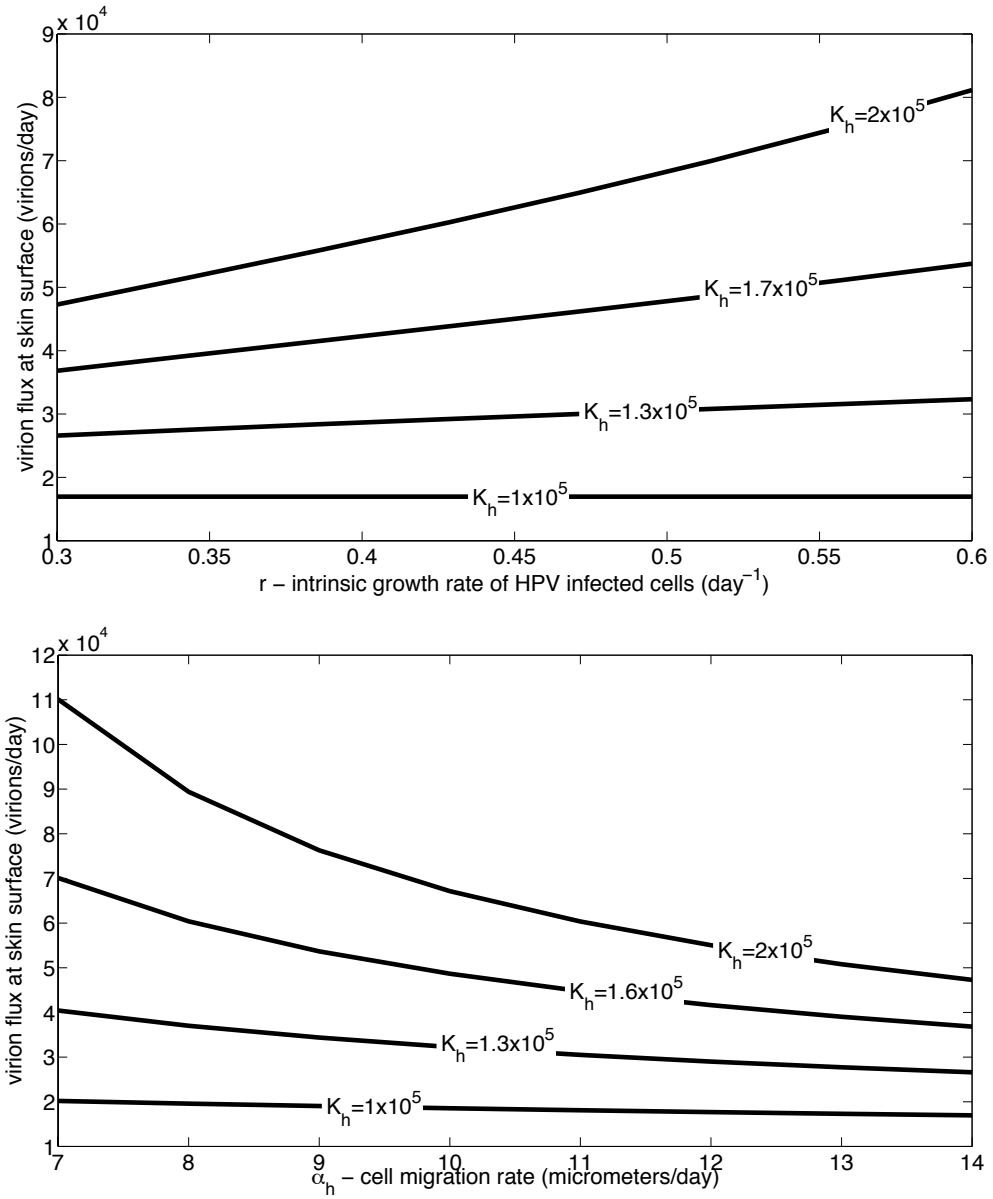


**Figure 6.3:** The effects of the model's ecological parameters on virion flux at the skin surface. The top panel shows the effect of the interaction of cell carrying capacity ($K_h$) and intrinsic growth rate ($r_h$) on virion flux. The bottom panel shows the effect of the interaction of cell carrying capacity ($K_h$) and cell migration rate ($\alpha_h$) on virion flux. Each line is associated with a value of infected cell carrying capacity. These values are given in units of cells/mm$^3$.

### **6.3.2** *Evolutionary Dynamics of HPV Lesions*

We use the second model to explore the role of somatic evolution in the progression of lesions. In order to do so, we start with a steady state HPV infection (LSIL), and assume that mutation has initiated a small population of autonomous and evolutionarily competent cells. We demonstrated with model 1 that if HPV only infected migrating cells, the infection was cleared. Thus, here we first investigate the effect of migration on the population of evolving cells. Figure 6.4 shows the difference between an evolving population of cells that migrates (top) and one that has lost the ability to migrate (bottom). Analogous to natural HPV infection, if the evolving cells migrate, then they are naturally cleared from the tissue within 40 days. However, if they do not migrate, they are able to form a dense tumor over time that spreads throughout the tissue.

During lesion progression researchers have observed that with time HPV's late gene expression and associated virion production attenuate until the productive phase of the viral life cycle is no longer supported (Middleton et al. 2003). Our model predicts the same qualitative pattern. Figure 6.5 shows the dynamics of HPV infected cells and virions as the tumor shown in the lower panel of figure 4 develops. As the tumor increases in density, the HPV infected cells are outcompeted and their abundance greatly diminished with time. Since there are less HPV infected cells, virion production also decreases with time. By just short of a year, virion production is nearly eliminated.

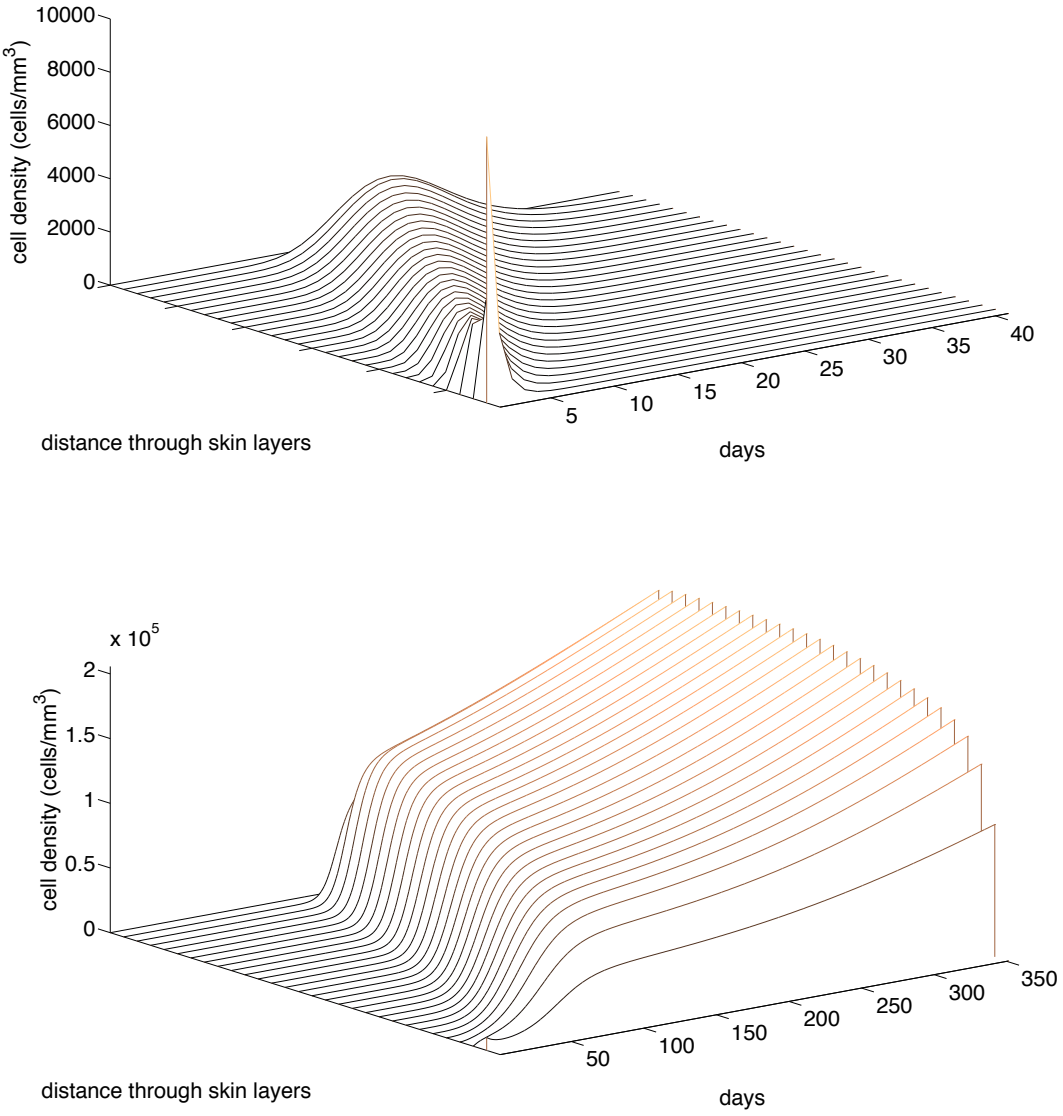


**Figure 6.4:** Population dynamics of autonomous evolving cells. The top panel shows evolving cells that migrate. The bottom panel shows evolving cells, which have lost their ability to migrate.

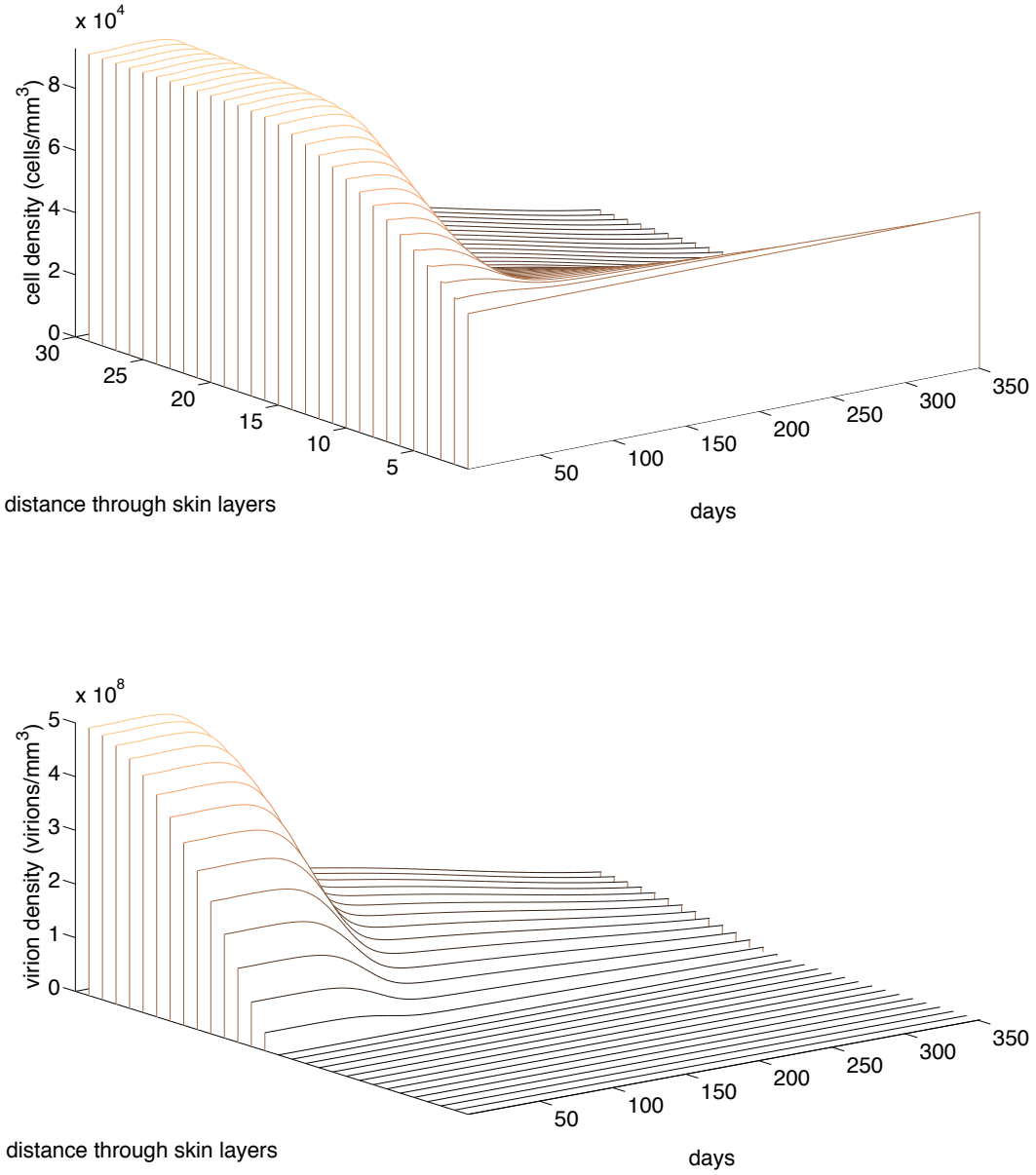


**Figure 6.5:** Dynamics of HPV infected cell and virion density as the evolving cells form a tumor. This is the same simulation as that shown for the evolving cells, which do not migrate in the bottom panel of figure 4. The top panel shows the dynamics of all HPV infected cells. The bottom panel shows the virion dynamics.

## 6.4 Discussion

In this paper, we use mathematical models to explore the ecology and evolution of HPV lesions. Our theoretical study makes two main contributions to the study of HPV related disease. First, we demonstrate the links between HPV infection and basic ecological parameters governing cellular population dynamics. Second, we highlight the role of somatic evolution in the progression of HPV lesions.

We argue that adaptive evolution plays a central role during lesion progression. Many of the effects of HPV proteins that have been studied likely contribute to initiating a population of autonomous cells. However, researchers have overlooked the connection between cell ecology and HPV infection. We show how modulation of ecological parameters is beneficial to HPV in terms of the number of virions shed from the surface of the skin per day. Because HPVs genes encode ecological strategies, which make HPV good at forming skin tumors, these genes are coopted and expressed by the autonomous cells. We show that once an autonomous population of cells is generated, these cells can further modulate HPV's ecological strategies through evolution by natural selection. Our model then predicts that the tissue changes observed from LSIL to HSIL are the result of evolving cells outcompeting HPV infected cells.

### **6.4.1** *The ecology of HPV lesions*

HPV infection has obvious effects on cell population dynamics. HPV might modulate cell population dynamics to compensate for it's non-lytic life cycle. Expanding the population of initially infected cells increases lesion size and the number of cells producing infectious virions. We used our model to explore the effects of modulating

basic ecological parameters on virion flux. We found that increasing the maximum tissue density at which cells could divide had a strong direct effect on virion flux. Thus we predict that HPVs early proteins are involved in decreased sensitivity to spatial crowding and low resources. HPV's E5 protein is known to increase the number of epidermal growth factor receptors on the cell surface (Straight et al. 1993). The E5 protein has also been shown to impair cell-cell communication (Oelze et al. 1995). This could be a way for HPV infected cells to ignore antigrowth signals from other cells. We also found that maximum cell division rate increases virion flux in combination with increases in the maximum tissue density at which cells divide. Interestingly, we found that decreasing cell migration rate has a weak effect on its own, but a strong effect in combination with increasing the maximum tissue density at which cells divide. There is some evidence that HPV early proteins modulate cell movement. Kivi et al. (2008) showed that HPV 16 E5 increased cell motility. Similarly, Boulenouar et al. (2010) demonstrated that HPV 16 early proteins increased motility in trophoblastic cells. However, it is unclear what the effects of increased cell motility are on directed cell migration to the skin surface. As our model shows, decreased migration is a potential strategy used by HPV to increase virion flux at the skin surface.

Empirically, the effect of HPV proteins on cell population dynamics could be revealed using raft skin cultures infected by HPV. Ecological models such as the one developed in this paper could be fit to the empirical data. Normal keratinocytes could be used as a control against which different types of HPV could be compared. The specific effect of HPV proteins on cell dynamics could be discerned through the use of genetically modified HPV episomes.

**6.4.2** *Evolution in the progression of HPV lesions*

HPV lesions progress to cancer, but lesion progression has not been framed in an explicit evolutionary context. Conceptual models put forward by others (e.g. zur Hausen 2000, Doorbar 2006, Snijders et al. 2006) include mutation, but do not include natural selection. Thus, they represent a molecular approach and not an explicit evolutionary approach.

We believe that lesion progression beyond LSIL is better understood in an evolutionary framework. Our model shows that somatic evolution can produce qualitatively similar tissue changes as those observed in the transition from LSIL to HSIL. We hypothesize that initiation occurs in LSIL and results in an autonomous population of cells. Our model predicts that these cells lose their tendency to migrate as part of the initiation step.

We predict that after initiation, cell and tissue changes occurring beyond LSIL are the result of adaptive evolution. Infected cells inherit and modify the ecological strategies encoded in HPV genes. Cells can coopt these strategies whether or not HPVs episome is integrated into the cells' chromosomes, since the viral genes are heritable in either form (not all HPV cancers have integrated viral genes). Our model predicts that as a population of cells evolve, they outcompete both normal and HPV infected cells. The lower abundance of HPV infected cells with time explains the observed decrease in the productive phase of the viral life cycle.

An alternative hypothesis for lesion progression is that the transition between LSIL and HSIL occurs as a series of initiation steps. This hypothesis is similar to the progression model proposed by Snijders et al. (2006). They present evidence for a

sequence of key genetic changes, which occur in the majority of HPV lesions during progression. Initiation is likely a multistep process. However, increases in chromosomal instability combined with other effects of HPV proteins (e.g. up regulation of telomerase) should speed up the initiation phase. Furthermore, it is unclear whether steps during the initiation phase lead to increases in cell populations, which is more tightly associated with phenotypic ecological advantages during adaptive evolution.

Lee et al. (2008) showed that phenotypic changes consistent with adaptation occurred throughout the progression of HPV lesions. GLUT1 expression increases during early progression, presumably as an adaptive response to hypoxia. Later on in progression, CAIX expression increases, presumably as an adaptive response to acidosis. However, their study does not specifically link these phenotypes to genes, and so it remains unclear whether these changes represent phenotypic plasticity of cells or intergenerational evolutionary change.

In conclusion, we believe that the ecological function of HPV's proteins contributes to the ecological success of cancer cells during adaptive evolution. We present a very simple model of evolution. Admittedly, the process is more complex. Nevertheless, this simple model provides an explanation of the tissue changes that occur during the initial progression of HPV lesions. The reductionist approach on the other hand identifies a multitude of genes and proteins, which are involved in progression. But there is no specific overarching conceptual framework in which to fit them. We believe that evolutionary theory can provide a conceptual framework to better understand the role of these molecules. This framework should include, mutation, initiation, and natural selection.

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## Appendix A

A zero net growth isocline (ZNGI) represents all combinations of resource abundances required for a consumer to subsist (Tilman 1980). Solving for ZNGIs generally requires setting per capita growth rates equal to zero and solving in terms of a particular resource's abundance. Here we show how to derive a ZNGI in the special case of a polymorphic species with a constant switching strategy in our food web without cannibalism. We start by setting the per capita growth rate of Jekyll to 0:

$$\frac{dJ}{Jdt} = \frac{b_{rj}a_{rj}R}{1 + a_{rj}h_{rj}R} - m_j - c + cq = 0$$

Let $q = H/J$ be the ratio of Hyde to Jekyll abundance. The equilibrium point of the system will be characterized by some value of $q$. Next we solve for $q$ in the above equation:

$$q^* = \frac{(m_j + c)(1 + a_{rj}h_{rj}R) - b_{rj}a_{rj}R}{c(1 + a_{rj}h_{rj}R)}$$

Then we substitute $q^*$ into the per capita growth rate of Hyde and set the equation to zero:

$$\frac{dH}{Hdt} = \frac{b_{ph}a_{ph}P}{1 + a_{ph}h_{ph}P} - m_h - c + \frac{c}{q^*} = 0$$

Finally, we solve the above equation for $R$ to get the ZNGI in the state space of $R$ vs. $P$:

$$R^* = \frac{(c + m_j)\left(c + m_h - \frac{b_{ph}a_{ph}P}{1 + a_{ph}h_{ph}P}\right) - c^2}{c^2 a_{rj}h_{rj} + (a_{rj}b_{rj} - a_{rj}h_{rj}(c + m_j))\left(c + m_h - \frac{b_{ph}a_{ph}P}{1 + a_{ph}h_{ph}P}\right)}$$

## Appendix B

Following Cohen (2009), we use a discrete function (equation 1) to describe mutation with regard to a continuous phenotypic trait ($w$). The $B$ function (equation 2) describes the per capita birth rate of a particular phenotype. We use 2nd order Taylor series approximations of the terms in equation 1 to convert the discrete equation into a continuous approximation. Equation 3 shows the 2nd order Taylor series approximation to equation 1, which we use for the numerical solution of the PDEs.

$$M = \delta\left[ B(w+\Delta)e\left(w+\Delta\right) + B(w-\Delta)e\left(w-\Delta\right)\right] \tag{1}$$

$$B(w) = \max\left( 0, r_e\left( 1 - \frac{T}{K_e(w)} \right) \right) \tag{2}$$

$$M = \delta\left[ B\left(e + e_{ww}\Delta^2/2\right) + B_w e_w \Delta^2 + B_{ww}\Delta^2/2\left(e + e_{ww}\Delta^2/2\right)\right] \tag{3}$$

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