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# Hard and Soft Selection Revisited: How Evolution by Natural Selection Works in the Real World

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## Abstract

The modern synthesis of evolutionary biology unified Darwin's natural selection with Mendelian genetics, but at the same time it created the dilemma of genetic load. [Lewontin and Hubby's \(1966\)](#) and [Harris's \(1966\)](#) characterization of genetic variation in natural populations increased the apparent burden of this load. Neutrality or near neutrality of genetic variation was one mechanism proposed for the revealed excessive genetic variation. Bruce Wallace coined the term "soft selection" to describe an alternative way for natural selection to operate that was consistent with observed variation. He envisioned nature as presenting ecological vacancies that could be filled by diverse genotypes. Survival and successful reproduction was a combined function of population density, genotype, and genotype frequencies, rather than a fixed value of the relative fitness of each genotype. My goal in this review is to explore the importance of soft selection in the real world. My motive and that of my colleagues as described here is not to explain what maintains genetic variation in natural populations, but rather to understand the factors that shape how organisms adapt to natural environments. We characterize how feedbacks between ecology and evolution shape both evolution and ecology. These feedbacks are mediated by density- and frequency-dependent selection, the mechanisms that underlie soft selection. Here, I report on our

progress in characterizing these types of selection with a combination of a consideration of the published literature and the results from my collaborators' and my research on natural populations of puppies.

**Subject areas:** Reproductive strategies and kinship analysis, Conservation genetics and biodiversity

**Key words:** density-dependent selection, eco-evo dynamics, frequency-dependent selection, integral projection models, life-history evolution

## Preamble

My goal is to present a minority perspective of the relationship between ecology and evolution, which is in turn a perspective on how I think evolution often happens in the real world. Because this is a convention of geneticists, I will begin by rooting the presentation in ideas that were once mainstream in population genetics, then show that very similar ideas arose independently in the early development of evolutionary ecology. My goal is to begin on ground that is familiar and, I hope, of interest, then shift to terrain that better represents my home turf. At the same time, my goal is to present some overview of the relationship between theory and empirical science and the interaction between the 2.

I will begin with J.B.S. Haldane, who contributed to the birth of the concept of genetic load. Haldane's models and argument were a direct outgrowth of the modern synthesis integration of Mendelian genetics with Darwinian evolution. A consequence of the models is that genetic variation is expensive because there is 1 best genotype, which is the genotype with the highest fitness. The presence of alternative alleles and additional genotypes reduces population mean fitness (Haldane 1937). Haldane's concept of the difference between the fitness of the best genotype and the population mean fitness became what many later referred to as genetic load. From these premises, he inferred that adaptive evolution in the form of the replacement of 1 allele by another can only happen at the expense of the death of a large number of individuals (Haldane 1957).

Haldane considered in quantitative terms what must have happened when the carbonaria allele replaced the wild-type allele in natural populations of *Biston betularia*, then he imagined what it would be like if this same selection were simultaneously applied to 10 characters:

"Now, if the change of environment had been so radical that ten other independently inherited characters had been subject to selection of the same intensity as that for color, only  $(1/2)^{10}$ , or one in 1024, of the original genotype would have survived. The species would presumably have become extinct."

(1957: J. Genetics 55, p. 511).

From these considerations, Haldane argued that adaptive evolution must be a slow process of allelic replacement, 1 locus at a time, each replacement happening over a period of centuries. A consequence of the genetic load associated with allelic replacement is that there should be little genetic variation segregating in natural populations. The variation we see should be in the form of rare recessives that represent recent mutations and are maintained by mutation–selection balance or by heterozygous advantage. He argued, with reference to the fossil record, that the empirical evidence strongly supports his expectation that evolution is a slow process.

There were alternative perspectives on genetic variation and evolution before and after Haldane, but his was an influential perspective that some referred to as the "classical hypothesis" (Lewontin

1974). Lewontin and Hubby's (1966), Hubby and Lewontin's (1966), and Harris's (1966) results represented the beginning and end of the notion that genetic variation in natural populations was as limited as implied by Haldane's models. At the same time, they created a new dilemma, which was how to explain the unexpected abundance of genetic variation in natural populations. Lewontin and Hubby (1966) considered neutrality, heterosis and mutation–selection balance as possible explanations, but none seemed to neatly explain the patterns of their data. Nevertheless, their results revealed an embarrassingly large amount of variation that demanded some explanation. A number of plausible explanations followed. The one that interests me today is Bruce Wallace's proposal of soft selection.

Bruce Wallace codified the dichotomy of hard and soft selection, with some inspiration from earlier work affiliated with Dobzhansky [summarized in (Dobzhansky 1962)] and from his own experiments (Wallace 1975). Hard selection refers to the selection envisioned by Haldane. Different genotypes have constant differences in fitness in a given environment. Soft selection instead envisions the environment as having vacancies to be filled. Who fills them is a function of population density (the number of contenders for vacancies) and the identity and frequencies of the different genotypes competing for these vacancies. Wallace (1975) presented this idea with an analogy, which was the existence of deanships of faculties. All faculties have a dean as their leader and this position is always filled. We hope that it will be filled by an individual with deanly qualities, but it will be filled regardless of whether or not such a person can be found, sometimes to our regret. Likewise, the vacancies in nature will be occupied, but there are not absolutes that define who the occupiers will be. Who fills these vacancies will be a function of how many vacancies there are, how many individuals there are to fill them and what the genotype frequencies are in the candidate population. This combination of density-dependent and frequency-dependent selection could thus explain the abundance of genetic variation. Different theoretical treatments of this sort of process, some long preceding Wallace's definition of hard and soft selection (Levene 1953) show that genetic variation can be sustained in such circumstances.

Charlesworth (2013) presents a modern manifestation of the same dilemma in a paper entitled "Why we are not dead one hundred times over", this time from the perspective of genome-wide analyses of genetic variation. Such analyses reveal the possibility of millions of silent sites that appear to be under weak purifying selection. His models show that the predicted genetic load from purifying selection at so many sites would be unbearable, unless one invokes soft selection. If selection is indeed soft, then such variation can be sustained under purifying selection without our being dead one hundred times over.

The invocation of soft selection as an explanation for observed patterns of genetic variation offers a solution to the dilemma of hard selection, but at the same time presents a challenge. How does evolution by natural selection work in the real world? Specifically, how might we characterize soft selection in nature and how prevalent is this form of selection? This is where I will abandon the turf of population genetics and move on to my own turf, which is evolutionary ecology. I will begin by showing that ideas very similar to

Wallace's were being proposed at almost the same time, and at the same university (Cornell), but apparently independently of Wallace. The context of this second origin was the early development of evolutionary ecology.

## Orians, Pimentel, Hutchinson and the Birth of Evolutionary Ecology

The inception of evolutionary ecology as a named subdiscipline dates to a paper by Orians (1962). Orians was actually trying to recast competing answers to the question that had dominated the field of ecology for decades: "what determines the abundance and distribution of organisms?" The 2 competing answers were that regulation was determined by density-independent versus density-dependent selection. Orians adopted a rabbinical approach, which was to say that both answers are correct, but address different phenomena. He suggested that we subdivide ecology into "functional ecology" and "evolutionary ecology". Functional ecology would replace density-independent population regulation and embody a proximate answer to what determines abundance and distribution, which is that they are a function of physiology and the physical environment. If we want to understand abundance and distribution, then we must study the physiological tolerance of the organism and see how it maps onto the physical environment. Evolutionary ecology replaces density-dependent regulation and provides the ultimate explanation for abundance and distribution, which is that both of these quanta are shaped by how an organism interacts with members of its own species or other species and how it adapts to these interactions.

Two versions of evolutionary ecology were articulated in the early 1960's. Hutchinson (1965) presents one version in his famous essay "The Ecological Theory and the Evolutionary Play". He argued that ecology, the stage, presents a template. Organisms, the actors, evolve to fit a niche within that template. Ecology thus governs and shapes evolution.

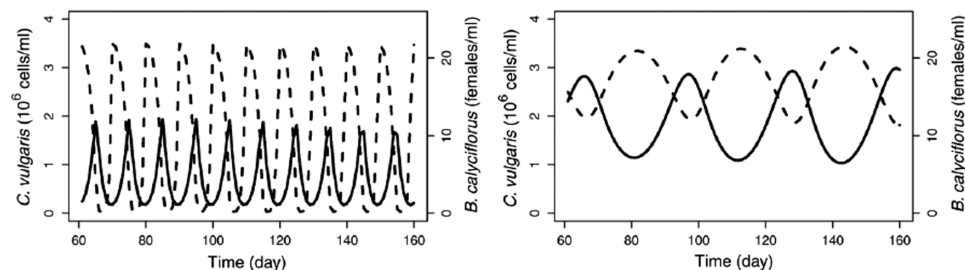
Pimentel (1961) presented an alternative perspective, which is that ecology and evolution are like 2 actors on the same stage, in a constant state of interaction with one another. He encapsulated this idea in what he called the "genetic feed-back mechanism": "Density influences selection; selection influences genetic make-up; and, in turn, genetic make-up influences density" (p. 65). Said differently, organisms can change their environment in a way that poses further selection upon themselves and hence further evolution. In a later paper, Pimentel (1968) generalized this feedback to a diversity of antagonistic interactions among organisms, such as between a host and parasite or pathogen, a predator and its prey, or among competitors. He also included frequency-dependent selection and

density-dependent selection as the key processes that govern the interaction between ecology and evolution.

Pimentel (1961, 1968) cites the rabbit–myxomatosis virus interaction in Australia as an example of this process. Rabbits were someone's Christmas gift to Australia. They were imported from Europe and released on Christmas day in 1859, within weeks of the publication of the Origin of Species. Decades later, the myxomatosis virus was imported from South America to control the rabbit outbreak that followed. There were successive waves of viral outbreaks, each separated by an interval when rabbit populations rebounded. The first wave of infection wiped out 97–99% of rabbits, the second 85–95%, and the third 40–60%. By the sixth only 25% of the rabbits were killed. These dampening oscillations were associated with the evolution of reduced pathogenicity in the virus and increased immunity in the rabbit. It is arguable that the virus shaped its own evolution through feedbacks between its virulence, impact on rabbit population density and probability of transmission to new hosts. More virulent strains were described as more efficient in converting rabbit tissue into virus, but killed off their hosts more quickly, reduced host population density and hence the viruses' chances of transmission. Less virulent strains preserved their hosts longer, facilitated higher host population densities and hence gained in the odds of being transmitted to new hosts. Pimentel described the rabbit–myxomatosis cycle as an interaction between ecology and evolution because it was shaped by the joint evolution of the host and pathogen, in conjunction with the expansion and contraction of the host populations and the effects of host population density on viral transmission. Remember that ecology in the early 1960's was defined by many as the study of factors that shape the abundance and distribution of organisms. Population density and distribution were thus primary components of ecology.

The key elements of Pimentel's perspective on evolutionary ecology are quite similar to those of Wallace's soft selection, which are that the interactions between ecology and evolution are driven by a combination of density-dependent and frequency-dependent selection. The 2 approaches differ in scope. Wallace addressed selection and the maintenance of genetic variation. Pimentel was not concerned with quantifying or explaining genetic variation. He was instead interested in evolution and how it interacts with ecology.

The historical aftermath of the emergence of Hutchinson's and Pimentel's perspectives of evolutionary ecology is that Hutchinson's perspective prevailed and Pimentel's languished. Hutchinson's view became what we now refer to as "evolutionary ecology". Pimentel's perspective remained, but lay in the background (Travis et al. 2013). It is now re-emerging, propelled by the development of new theory and some key experiments.



**Figure 1.** This figure is modified from the original presented as Fig. 1 in Yoshida et al. (2004). The figure depicts predator–prey oscillations in either the absence (left panel) or presence (right panel) of prey evolution. The left panel characterizes the oscillation of prey (the algae *Chlorella vulgaris*, dashed line) and predator (the rotifer *Brachionis calyciflorus*, solid line) when the chemostat (or simulation) is initiated with a single clone of prey. The right panel illustrates the same interaction when multiple clones of prey are present. If there are multiple clones, then evolution is possible via changes in the relative abundance of clones.

## Rotifers and Algae

Figure 1 illustrates a more modern manifestation of feedbacks between ecology and evolution and why they matter. The figure illustrates the results of a model, but is backed up by experimental studies of the interactions between the rotifer (*Brachionus calyciflorus*) and the algae (*Chlorella vulgaris*), performed in chemostats that enclose a model, 2 species ecosystem (Fussmann et al. 2000; Shertzer et al. 2002; Yoshida et al. 2003). This series of papers began with a study of the joint population dynamics of the interaction between these 2 species that revealed some unexpected results (Fussmann et al. 2000). The authors developed alternative models to explain these results (Shertzer et al. 2002), then performed the experiment to identify the model that represented the most likely explanation for the pattern (Yoshida et al. 2003). Figure 1 represents the output of the model, but also accurately represents the results of the experiment. The left-hand panel shows what a predator–prey oscillation looks like when there is only a single clone of prey and hence no evolution. The right-hand panel presents the same interaction, but with the added complication that it was initiated with multiple clones of prey. This added complexity makes evolution possible in the form of changes in the relative abundance of the different clones and hence the genetic composition of the population. The addition of evolution changes everything about the interaction. The period and amplitude of the cycles are different, as is the degree to which the peaks and troughs of the predator and prey cycles are offset from one another (Yoshida et al. 2003). The reason the dynamics of the interaction change is that the prey population consists of clones that are competitively superior, but more susceptible to predation, versus others that are competitively inferior, but resistant to predation (Yoshida et al. 2004). As rotifers increase in abundance and deplete the population of algae, they do so by selectively feeding on the competitively superior but susceptible clones. Selective predation causes the algae population to become dominated by predator resistant clones, which in turn causes the abundance of predators to decline. As predators decline, the competitively superior clones of algae once again increase in frequency, which recreates circumstances that allow the predator population to recover and resume growth. The cycles are thus the product of a complex mix of density cycles and the continuous evolution of the prey in response to frequency-dependent selection by the predator. Said differently, they are a manifestation of soft selection.

This brings me to a definition of what is now referred to as eco-evo feedbacks: such feedbacks occur 1) when the evolution of a participant in an ecological interaction causes a change in the nature of the outcome or 2) when an organism modifies its environment and hence the selection it experiences, causing it to evolve to a different endpoint from what is predicted had it not changed its environment. This perspective is fundamentally different from the Hutchinsonian version of evolutionary ecology. The Hutchinsonian perspective implicitly assumes that organisms do not evolve. It does so because the associated theory and empirical research treats organisms as constants. However, species are not constant. Furthermore, their rate of evolution can happen on time frames similar to the time frame of ecological interactions (Schoener 2011), which makes such interactions more amenable to empirical study. These conditions, and the potential impact of such feedbacks, raise the question “how prevalent are such interactions in nature?” The answer is that we do not know because little effort has been invested in characterizing them. A second, related question is “why should we care?” The reason we should care is that feedbacks between ecology and evolution can change the predicted outcome of how the organism will evolve and the outcome of ecological interactions.

## Guppies

### The Setting

Colleagues have been addressing the possible feedbacks between ecology and evolution in our ongoing studies of natural populations of guppies from the Northern Range Mountains of Trinidad. Our interest is in how guppies adapt to the large differences in the risk of predation they experience in natural high and low predation (LP) communities (Haskins et al. 1961; Endler 1978; Reznick et al. 1996). High predation (HP) communities tend to be found in the lower reaches of river drainages, where guppies co-occur with large predatory fish species. Waterfalls and rapids often exclude predators but not guppies or the killifish *Rivulus hartii*, from the upper reaches of these streams and thus create LP environments where guppies co-occur with just *Rivulus*. *Rivulus* is a less severe predator on guppies (Haskins et al. 1961; Endler 1978; Reznick et al. 1996).

The contrast between HP and LP communities is replicated in different rivers (Endler 1978). Genetic differences among rivers support the argument that at least some of them represent independent occurrences of guppies adapting to life with and without predators (Alexander et al. 2006; Willing et al. 2010). Nature thus provides the equivalent of a replicated experiment. These differences in predation have caused the convergent evolution of life histories (Reznick and Bryga 1996; Reznick et al. 1996) plus a diversity of other traits, including male coloration, behavior (Endler 1995), and neuromuscular performance (Ghalambor et al. 2004). HP and LP communities can often be found in close proximity to one another, separated by a waterfall, so that the physical environment is very much the same, even though the fish communities differ. Waterfalls sometimes exclude all species of fish, save *Rivulus hartii*. *Rivulus* is able to breach all barriers because they can disperse overland on rainy nights. Such discontinuities create the opportunity to treat streams like giant test tubes and experimentally manipulate a guppy's risk of mortality. We can decrease the guppies' risk of mortality by transplanting them over barrier waterfalls that separate HP communities from communities that only contain *Rivulus* (Endler 1980; Reznick et al. 1990; Reznick et al. 1997) or increase the risk of mortality by introducing predators over waterfalls that separate LP from HP communities (Reznick 1997). These experiments formally test the predictions of evolutionary theory plus demonstrate the rapidity of evolution. Guppies are also good organisms for laboratory research. We compare second generation descendents of wild-caught females in a common environment to enable us to make more direct inferences about genetic differences between them.

### The Results

Guppies from HP communities are younger and smaller at sexual maturity than those from LP communities. They produce more babies per litter. Individual offspring are smaller. HP guppies also devote more resources to each litter and have shorter intervals of time between successive litters (Reznick 1982; Reznick and Endler 1982). These results are demonstrable in wild-caught fish and in the laboratory reared grandchildren of wild-caught fish. They are also repeatable between the north and south slopes of the Northern Range mountains, which differ in the predator fauna (Reznick and Bryga 1996; Reznick 1996). The south slope rivers were once tributaries to rivers in mainland South America and have predators typical of the mainland (characins and cichlids). The north slope rivers never had a mainland connection and are instead colonized by predators derived from a marine environment (gobies and mullets). While the species identity of the predators is different, the presumed

impact of predators on a guppy's risk of mortality is the same. It thus appears that it is the differences in risk of mortality between HP and LP communities that is driving guppy life-history evolution.

All of these differences between HP and LP guppy populations in life-history traits are consistent with predictions derived from early and simple models of life-history evolution (Gadgil and Bossert 1970; Law 1979; Michod 1979; Charlesworth 1980), which predict how life histories will evolve in response to differences in age-specific risk of mortality. We knew from direct observation that some predators in HP communities target large, adult size classes of prey while *Rivulus* target small, immature size classes of prey. These models predict that a selective increase in adult mortality risk (HP communities) will favor the evolution of earlier maturity and increased investment in reproduction. Conversely, they predict that a selective increase in juvenile mortality risk (LP communities) will select for the opposite spectrum of life-history traits—delayed maturity and reduced investment in reproduction. Our introduction experiments confirmed these predictions (Reznick and Bryga 1987; Reznick et al. 1990; Reznick et al. 1997; Reznick 1997).

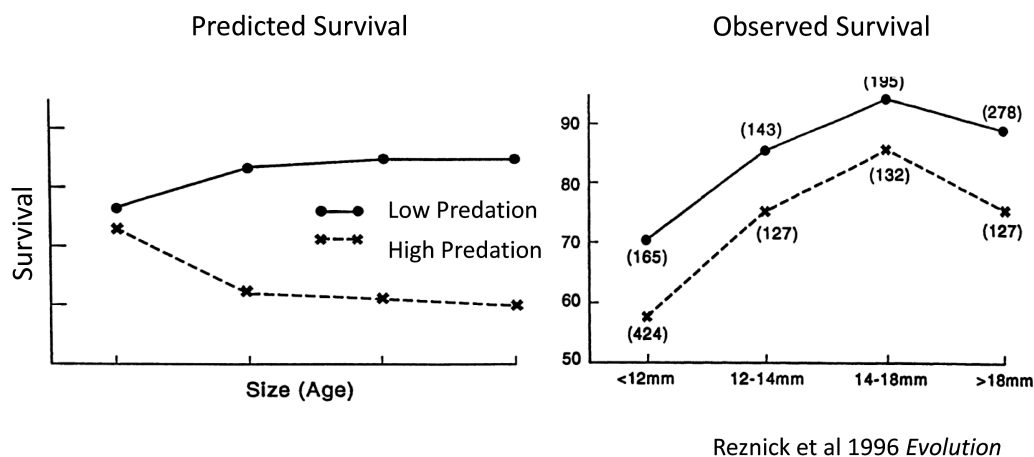
### Life was Good, Then...

The direct estimation of mortality rate was a missing link in the early phases of this research. Beginning in the late 1980's, we implemented mark-recapture methods to get direct estimates of mortality rates in natural populations. The expectation was that overall mortality rates would be higher in HP environments, but also that the differences would become magnified in larger/older size classes of fish. The implied interaction between predator community and size/age in mortality risk was critical for matching the biology of guppies to the theory we were using to predict life-history evolution because the theory modeled the effects of age-specific risks of mortality. Our results did indeed show that guppies from HP environments experience substantially higher mortality risks, but they did not show the predicted interaction between community and size class (Figure 2). We instead found an approximately equal difference in mortality risk across all size classes of guppies. The absence of the expected

interaction is critical. The same theoretical papers that we were using as a source of predictions had also addressed how life histories should evolve when the added risk of mortality was equal across all age classes. In that case, they predicted that the life history would not evolve. In the context of these models, some heterogeneity among age classes in mortality risk was necessary to cause life-history evolution. This was a dilemma because we already knew that guppy life histories evolve to different endpoints in HP and LP environments. There was clearly a mismatch between the models we were using as a conceptual framework and the reality of guppies in natural streams.

An important feature of these models is that they exclude any consideration of ecology save differences among populations in mortality risk. Populations were assumed to grow exponentially, meaning that there were no limitations of resources. In this context, fitness is equal to the intrinsic rate of population growth. Alternative models, also proposed in some of these earliest theoretical papers (Michod 1979; Charlesworth 1980) and developed more fully later (Charlesworth 1994; Brommer 2000), added ecological complexity. A simple alternative is for there to be density-dependent population regulation in the prey, although many other alternatives are possible (Abrams and Rowe 1996). Models that include such added complexity make it possible for the observed patterns of mortality to be reconciled with the observed patterns of life-history evolution. These models also suggested that we needed to consider the comparative ecology and population dynamics of guppies in LP and HP environments.

We performed such a comparative study on natural LP and HP communities (Reznick et al. 2001). Guppies from LP environments have 4–5 times higher biomass per unit area or volume of stream. The 2 types of communities also have different size/age structures; HP populations are dominated by small, young fish while LP environments have a more even representation of age and size classes. These differences in structure are attributable to the combined effects of the environment and evolved differences in life histories. HP guppies sustain higher mortality rates but also mature when younger and produce more babies (Rodd and Reznick 1997). Guppies from HP environments also have higher growth rates (Reznick et al. 2001; Reznick and Bryant 2007), which are most likely attributable to



**Figure 2.** Predicted and observed size-specific mortality in guppies. For our results to be consistent with the theory used to predict the evolution of guppy life histories, there must be an interaction between size/age class and risk of mortality, such that the risk of mortality increase faster with age in high predation environments than low predation environments. The left hand panel illustrates one such predicted interaction profile. The y axis depicts the probability of survival while the x axis depicts 4 size(age) classes, with the smallest(youngest) on the left and progressively older(larger) individuals to the right. Our results (right panel) confirm that guppies sustain higher mortality risk in high predation environments, but without the predicted interaction between size and predation. Here, the y axis depicts the probability of recapture after 12 days and the x axis depicts 4 different size classes of fish. Guppies have indeterminate growth. Size is well correlated with age. The results in the right panel are a composite of 14 mark-recapture studies, 7 each in high and low predation environments.

their having higher resource availability. Growth rate differences are small or absent when these fish are reared on controlled rations in the laboratory (Arendt and Reznick 2005).

The differences between HP and LP populations in population density, size structure, and growth rates might be caused by the indirect consequences of predation. Reduced predation in LP environments allows guppy populations to proliferate, followed by reduced per capita food availability. The combination of our estimates of mortality rates, our inferences from theory about the possible evolutionary consequences of density regulation and our empirical evidence for indirect consequences of predation suggest that more than predator-induced mortality is shaping guppy life-history evolution. Our first consideration was to ask whether density regulation and density-dependent selection were indeed present in our populations.

### Do Guppies Experience Density-dependent Selection and Evolution?

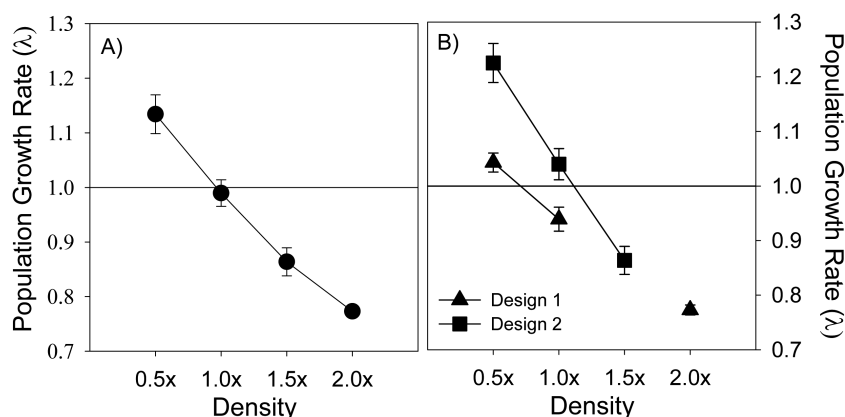
We first assessed and characterized density regulation in natural LP populations by experimentally manipulating density, then quantifying the life-history response (Reznick et al. 2012; Bassar et al. 2013). We performed these experiments in streams that have a riffle-pool structure. Pools do not have a linear flow of water from up to downstream. Pools are separated from one another by riffles, which have a steeper gradient and through which water flows at a higher rate and in a straight line. We discovered that guppies aggregate in pools and have low migration rates from pool to pool (Reznick et al. 1996). Because guppy populations within pools are relatively stable, we used individual pools as our unit of replication in experiments in which we either increased, decreased, or maintained the ambient population density. If guppy populations at ambient density are subject to density regulation, then reduced density should cause demographic changes that result in an increase in population growth rate. Increased density should cause demographic changes that result in decreased population growth rate. These responses would return the population to ambient density. Our projection of population growth rate in the

ambient density treatments was very close to one, meaning that these populations should remain stable for the near term. Increased density causes the rate of population growth to be negative while decreased density causes it to be positive (Figure 3). These changes in projected population growth were caused by effects of density on growth rates, reproductive allocation, and mortality rate (Reznick et al. 2012). Guppies in LP environments are indeed density regulated.

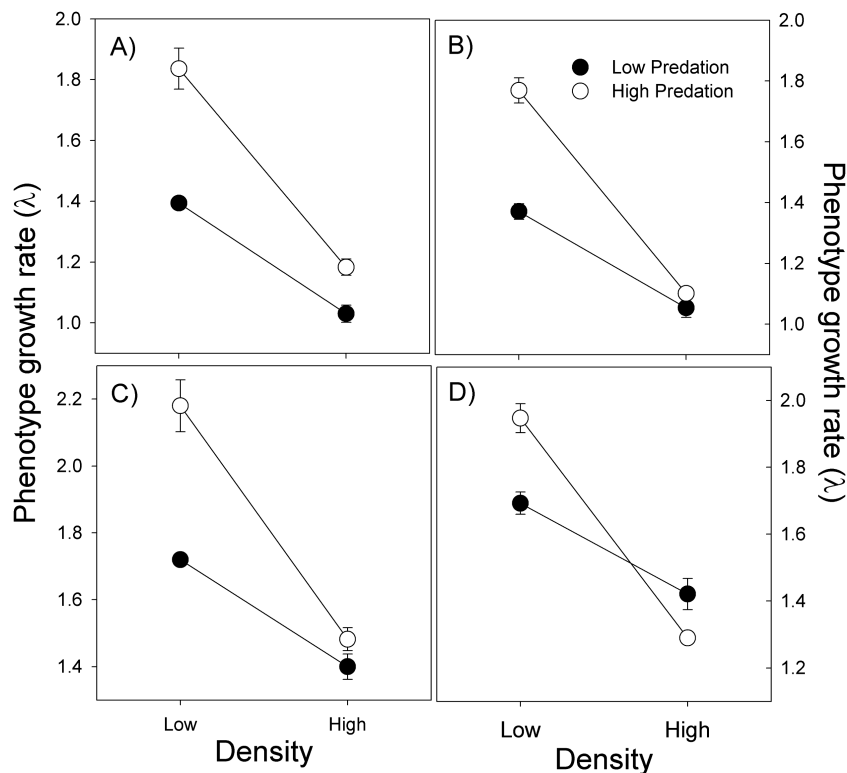
We addressed whether or not guppies in LP environments are adapted to high density with experiments performed artificial streams, built alongside a natural stream in Trinidad (Bassar et al. 2010; Bassar et al. 2012; Bassar et al. 2013). Our experiment included a 2-way factorial cross of guppies derived from an HP or LP localities introduced into the stream at either high or low population densities. HP guppies have higher fitness than LP guppies when at low population densities, but the difference disappears at high population densities (Figure 4). This interaction suggests that adaptation to density has played a role in shaping the how guppies adapt to LP environments because guppies from LP environments are less sensitive to density. The high density of guppies in LP environments has a sufficient impact on some aspect of its ecology for it to feed-back on and cause evolution in guppies, as originally postulated by Pimentel with his genetic feedback hypothesis. However, the results also tell us that such adaptation is not sufficient to explain the evolution of the LP phenotype. The reason is that density only makes HP and LP guppies equal in fitness. For the LP phenotype to displace the HP phenotype, it must have higher fitness.

### Guppy–*Rivulus* Interactions

An additional factor that might come into play to explain the evolution of the LP phenotype is the interaction between guppies and *Rivulus*. When guppies and *Rivulus* co-occur in headwater streams, in the absence of predators, they are both found at much higher population densities than when they co-occur with predators further downstream (Gilliam et al. 1993). Their high population densities create the possibility of strong interspecific interactions and



**Figure 3.** Density-dependent regulation—results of density manipulation experiments. Each replicate include 3 pools. All guppies were collected from each pool, individually marked, then released back in to the pools at either ambient density (1x), half ambient density (0.5x) or increased density (2x in Design 1, the first series of experiments and 1.5x in Design 2, the second series). These density treatments are represented by the x axis in each panel, with reduced density (0.5x) on the left and increased density (1.5 or 2.0x) on the right. Each fish in the experiment was measured marked before reintroduction and again at the end of the experiment, enabling us to estimate individual growth rates. Mortality rate was estimated from recapture probability. We preserved all fish at the end of the experiment, then dissected the females to characterize the rate of offspring production. We summarized the demographic data with an integral projection model that provided a point estimate of the rate of increase in population size, which is in turn a point estimate of the relative fitness of each population. The y axes in this figure represent these estimated values of population growth rate for the different treatment groups. A value of 1.0 implies a stable population size over the course of the experiment. Values >1 correspond to increasing population size and values <1 correspond to decreasing population size. Error bars are  $\pm 1$  standard deviation (Fig. 1 from Bassar et al. 2013). (A) shows the results when the two designs were combined. (B) gives the results when each design is analyzed separately.



**Figure 4.** Density-dependent evolution—results of the factorial comparison of the fitness of HP and LP guppies reared in artificial streams at high and low population densities. We constructed 16 artificial stream channels along side a natural stream and established flow by diverting flow from a spring that normally fed the natural stream. We filled the artificial streams with natural stream substrate (sand and gravel) and colonized them with invertebrates from the neighboring stream. Density and size structure of introduced guppies were parameterized with estimates from comparative ecological studies of natural streams (Rodd and Reznick 1997; Reznick et al. 2001); low density corresponded to the mean density in HP environments and high density corresponded to mean density in LP environments. We replicated the experiment by first running it with guppies derived from paired HP and LP localities in the Aripo River, then with guppies derived from HP and LP localities in the Guanapo River. The results presented here are the population growth rates (y axis) at high and low population densities (x axis), as estimated from the application of integral projection models to the demographic output of each channel, which includes survival, individual growth and reproduction, as estimated from the dissection of the fish preserved at the end of the study. The upper panels represent calculations using the survival estimates from the density manipulations in natural streams. The lower panels are based on the survival estimates in this experiment. (A) and (C) represent the results for guppies derived from HP and LP environments in the Guanapo River. (B) and (D) represent results for guppies derived from HP and LP environments in the Aripo River (Figure 3 from Bassar et al. 2013).

coadaptation to each other. When *Rivulus* are found by themselves, upstream of barriers that exclude guppies, they are found at higher population densities than when guppies are present, which suggests that guppies reduce *Rivulus* abundance (Gilliam et al. 1993; Walsh et al. 2011).

Matt Walsh showed that guppies drive the evolution of killifish and do so via indirect effects (Walsh and Reznick 2009; Walsh and Reznick 2010; Walsh et al. 2011; Walsh and Reznick 2011). Guppies reduce the abundance of *Rivulus*, most probably by eating and/or competing with juveniles (Fraser and Lamphere 2013). *Rivulus* from LP environments have higher growth rates than those from *Rivulus*-only environments upstream. When Walsh transplanted individually marked *Rivulus* from the *Rivulus* only environment into the LP environment downstream, their growth rates accelerated to match those of the residents, suggesting that the higher growth rates were a simple consequence of higher per capita food availability (Walsh et al. 2011). When he reared the grandchildren of wild-caught *Rivulus* in a laboratory setting, he found that *Rivulus* from LP environments had higher fitness than those from *Rivulus*-only environments when food was abundant, but lower fitness when food was limited (Walsh and Reznick 2010; Walsh and Reznick 2011). This food by population interaction suggests that *Rivulus* from LP environments have

adapted to high food availability, which is an indirect consequence of guppies reducing their population density and increasing per capita food availability.

We have completed artificial stream experiments in which we quantified the relative fitness of HP and LP guppies when kept in artificial streams with *Rivulus* derived from either *Rivulus*-only or LP environments (Bassar et al., in preparation). The IPM analyses of the guppies in these experiments show that LP guppies have higher fitness (higher population growth rates) than HP guppies when they are kept with *Rivulus*. The fitness advantage of LP guppies is greater still if the *Rivulus* were derived from localities where they co-occur with guppies than if they were derived from localities where *Rivulus* was the only species of fish present. This difference suggests that there has been some form of ecological displacement between guppies and *Rivulus*. This experiment was replicated, once with fish derived from the Aripo River and once with fish from the Quare River, both times with the same results.

Guppies and *Rivulus* thus impose selection on each other and both have evolved in response to that selection. There are thus multiple possible forms of feedback between ecology and evolution. One is density-dependent selection. For population density to shape local adaptation, there is likely to be some mediating influence of guppies

on the environment, perhaps via their impact on resource availability. A second is interspecific interactions with *Rivulus*. These species have reciprocal effects on each others' abundance and distribution that shapes how each of them are adapting to life without predators in headwater streams. It is this interaction that tips the balance in favor of the LP phenotype over the HP phenotype.

## How Does Evolution Shape Ecology?

Each of the above experiments suggests a feedback between ecology and evolution, but both admit diverse possible routes for these feedbacks to occur and neither study characterizes the feedbacks. In the laboratory study of the interaction between rotifers and algae (Yoshida et al. 2003), performed in chemostats, all features of the environment could be controlled. In our case, the linkage between guppies and density or guppies and *Rivulus* lies in the complexity of a natural ecosystem. We are trying to elucidate the nature of these linkages as part of our artificial stream experiments (Bassar et al. 2010; Bassar et al. 2012; Bassar et al. 2013), but also in the context of experiments done in natural streams. The experiment summarized in Figure 4 included a fifth treatment—streams with no guppies. A comparison of the “no guppy” treatment with the mean of the 4 treatments that included guppies yields an estimate of guppy impacts on the ecosystem. A factorial comparison of HP versus LP guppies, high versus low population density, and the interaction between guppy phenotype and population density further characterizes how the adaptation of guppies to different environments affects their impact on ecosystem structure.

Our dependent variables were chosen to characterize the sources of nutrient input to a stream ecosystem, then how this input is channeled through the different components of the community. Input comes in the form of primary production in the stream and from terrestrial matter, such as leaves, that falls into the stream then is broken down by leaf-shredding insects and microbes. The presence of guppies was associated with a depletion of the standing crop of algae and invertebrates. Guppies reduced area-specific gross primary production and community-wide respiration, but increased mass-specific gross primary production (Bassar et al. 2010). Guppy effects on the abundance of algae and invertebrates are more pronounced at high population densities than low population densities. This result suggests that the density-dependent evolution could be mediated through the way guppies deplete the environment of food resources. These results also satisfy a pre-requisite for feedbacks between ecology and evolution to occur because they prove that guppies can change the structure of their ecosystem.

HP and LP guppies differ in how they impact the environment. HP guppies depleted invertebrate abundance to a greater degree and algal abundance to a lesser degree than LP guppies. At the end of the 4-week experiment, we preserved the fish and analyzed their stomach contents. We found that HP guppies consumed more invertebrates and less algae than LP guppies. We obtained the same result when we compared the stomach contents of HP and LP guppies collected from natural streams (Zandonata et al. 2011). The differences in the impact of HP and LP guppies on the environment may be mediated by differences in their dietary preferences. HP guppies fed selectively on high quality prey, meaning prey that had lower C/N ratios and hence higher protein content. LP guppies instead consume resources in proportion to their abundance in the environment (Zandonata et al. 2011). One possible mechanism for density-dependent evolution might thus be that guppies adapt to their depletion of high quality food resources in LP environments by becoming less selective consumers and relying on a lower quality diet.

We thus have strong evidence for the direct effect of guppies on the ecosystem. In the absence of predators, guppies deplete the ecosystem of algae and invertebrates. It appears that they adapt to this change by becoming generalist consumers. There is some evidence that this change was facilitated by the evolution of their skull morphology (Palkovacs et al. 2011). These added results are putting meat on the bones of Pimentel's proposed feedback between ecology and evolution because they are beginning to reveal the mechanisms that underlie the ongoing interaction between ecology and evolution.

## Experimental Evolution

We are also pursuing a study of eco-evo interactions prospectively, in the context of a replicated experiment, performed in natural streams. I will only introduce this study today just to give you a sense of where we are going with the system. Our study sites are 4 headwater streams in the Guanapo River. Each shares the property of being separated from the main river by one or more waterfalls that exclude all fish species save *Rivulus*. In each we defined a stretch of stream, bounded by waterfalls on either side, as a guppy introduction site. An additional stretch upstream of the guppy introduction site was defined as our control. We initiated an individual-based mark-recapture study of the *Rivulus* and began monitoring features of the stream ecosystem (invertebrates, algal standing crop, benthic organic matter, stream metabolism) in the control and introduction sites a year before guppies were introduced, then continued for 4 years after the guppy introduction. We initiated the introductions with individually marked guppies, conduct censuses once a month and mark all new recruits. We collect scales from the founders and all new recruits to provide a source of DNA so that we can reconstruct pedigrees and quantify individual reproductive success, plus facilitate future studies of the genetics of adaptation. In this setting, we are following the evolution of guppies and *Rivulus* and characterizing how the ecosystem changes over time. The data stream is a complex time series that will make it difficult to define cause and effect relationships. Our intent is to integrate this work with our ongoing series of experiments in artificial streams and to define cause and effect with appropriate, short-term factorial experiments similar to those reported above.

## Progress

The impact of introduced guppies on *Rivulus* became apparent within a year of the introduction. *Rivulus* abundance has declined and mean body size has increased in all 4 streams (Fraser and Lamphere 2013) because guppies are reducing *Rivulus* recruitment. Quantifying the impact of introduced guppies on other features of the stream ecosystem has been more elusive. A clear distinction between a natural stream and our artificial streams is the large impact of seasonal rainfall. The flashes of high water flow that follow heavy rains often scour streams of invertebrates and algae, so they are in frequent state of community re-establishment (Kohler et al. 2012). It is only during the dry season that we might see a sufficient period of stability for guppy impacts on the ecosystem to become apparent. At these times, we have seen significant reductions in the abundance of invertebrates and algae, but not in all streams in all dry seasons. Ecosystems assessments remain a work in progress.

We are following guppy evolution as change in the mean phenotype of the population over time, as inferred from photographs that enable us to quantify male size at maturity, body shape, and aspects of coloration. We infer the evolution of a full spectrum of life-history traits from laboratory common garden experiments, as in prior studies. Pedigree reconstruction enables us to also study evolution

as variation in individual reproductive success and the association between reproductive success and aspects of the phenotype, as in the work done on organisms like the Soay Sheep or red deer (Foerster et al. 2007; Ozgul et al. 2009; Coulson et al. 2010). This again is a work in progress, which is currently represented in the literature by 2 publications in which we integrate the pedigree with the mark recapture. In one, we make inferences about how variation in early growth history affects future reproductive success. In the second, we show that males continue to sire offspring for at least 10 months after their death because their sperm live on in longer lived females (Auer et al. 2012; Lopez-Sepulcre et al. 2013).

## Conclusions

I opened with a consideration of the similarity between Wallace's soft selection and Pimentel's "genetic feedback mechanism", both of which promoted the importance of density-dependent and frequency-dependent selection, but for different reasons. Wallace sought to explain the abundance of genetic variation in natural populations. Pimentel was instead interested in how ecology and evolution interact with one another to shape adaptation and population dynamics. The importance of Pimentel's vision for evolutionary ecology is that the existence of such interactions can yield fundamentally different results from what one would predict when interactions between ecology and evolution are ignored. My interest is in characterizing the importance of these interactions, and of soft selection, in shaping natural ecosystems.

## Interplay Between Theory and Empiricism

Our work thus far fulfills Pimentel's ideas for defining the relationship between ecology and evolution. LP guppy populations are density regulated. The interaction between density and the relative fitness of HP and LP guppies is a signature of density-dependent evolution. Guppies are capable of altering ecosystem structure in a way that can impose selection on themselves because they deplete resource availability. There is also a signature of a cascading effect of guppies on other components of the ecosystem that include changes in the population dynamics of *Rivulus*, then co-evolution between *Rivulus* and guppies. The aggregate of all of these results suggests that, when guppies are released from predation in LP environments, their increase in abundance causes changes in ecosystem and in the kind of selection they experience. Their adaptation to these settings includes adaptation to their own impact on the ecosystem. We have not yet fully characterized the impact of guppies on the ecosystem of natural streams or how these impacts reshape the selection on and subsequent evolution of guppies and *Rivulus*. Fulfilling these goals extends Pimentel's vision by placing it in the context of the complexity of natural communities. We have also not yet characterized the role of frequency-dependent selection, which is a necessary step if we are to equate Pimentel's genetic feedback mechanism with soft selection. Our discovery and characterization of the evolutionary interactions between guppies and *Rivulus* adds interspecific frequency dependence to the possible venues for frequency dependence to act.

## How General Are Our Results?

My ultimate goal is not to detail guppy evolution, but rather to promote Pimentel's vision of soft selection as a new and different way of thinking about the relationship between evolution and ecology. If interactions between ecology and evolution are a prevalent feature of natural ecosystems, then there is the promise that we can improve ecology and evolution as predictive sciences if we can master how

to characterize them. It thus becomes important to consider how common such feedbacks might be and to identify good targets for future study so that we can make progress in characterizing them. Such work could also lead to the integration of ecosystems ecology with population/evolutionary ecology, because ecosystems ecology provides concepts and tools for detailing the diverse pathways that lie between evolving populations of interacting species. A second consequence of our results is that they show that the properties of an ecosystem are evolved properties in the sense that the local adaptations of key residents can play a significant role in shaping the structure and processes of ecosystems.

## Where Else Are Such Feedbacks Known to Occur?

The list of research programs in natural ecosystems where investigators have set out to explicitly characterize contemporary interactions between ecology and evolution is short. This list can be lengthened if we include research programs that fulfill the ideals but are known under different labels. Here, I offer 2 exemplars.

Megan Duffy and collaborators have characterized how the intensity and persistence of epidemics are shaped by the evolution of host resistance, genetic tradeoffs between host resistance and fecundity, habitat productivity, predation, competition and the mode of transmission (Duffy and Hall 2008; Duffy et al. 2009; Duffy et al. 2012; Penczykowski et al. 2014). An important message of this complex body of work is that evolution is well integrated with ecological interactions and can significantly change their outcome. Furthermore, they show, as did Yoshida et al. (2003) that the inclusion of evolution in models of population dynamics significantly improves the ability of models to predict the outcome of these ecological interactions (Duffy and Hall 2008).

Barry Sinervo and collaborators' work on rock-paper-scissors interactions in *Uta stansburiana* is best known for its characterization of sexual conflict and social interactions, but serves well as an exemplar of feedbacks between ecology and evolution. This phenomenon is really a combination of rapid, cyclical evolution driven by frequency-dependent selection on male phenotypes (Sinervo and Lively 1996) and density-dependent selection on female phenotypes (Sinervo et al. 2000). Underlying drivers of the phenomenon include population size, territory size, and resource availability within territories. Sinervo et al. (2007) document similar cycles in *Lacerta vivipara*, cite evidence for such interactions in a diversity of other organisms and argue that this type of phenomenon could be widespread. It could explain the sustained variation in sexually selected traits that is characteristic of many organisms.

## Where Can We Find the Best Opportunities for Future Study?

A reasonable target for study includes any species that, like guppies, can be found in diverse ecological settings where there may be natural variation in the intensity of density regulation. Targets also include any circumstance in which we see strong ecological interactions, such as those associated with trophic cascades or keystone species. Trophic cascades and keystone species are well known from a diversity of ecosystems (Estes et al. 2013). While their ecological consequences are well understood and have been characterized in many ecosystems, their evolutionary consequences have not been considered. To seek evolution and interactions between ecology and evolution, one would have to focus on species found in communities with and without the protagonists that are the keystone species

and/or create the trophic cascade. What happens to these innocent bystanders when the keystone is removed? How do they change the ecosystem and how might these changes feedback and change the selection they experience, causing them to evolve. Think of mussels in the absence of starfish or urchins and kelp in the absence of otters.

Feedbacks between ecology and evolution can also illuminate applied aspects of human impacts on the environment. For example, Estes et al. (2011) vividly illustrate what happens to ecosystems as humans selectively eliminate apex predators. Eliminating predators causes a cascade of events similar to what happens when guppies invade previously guppy-free headwater streams. In the absence of predators, some prey species proliferate, then restructure their ecosystems. One famous example is the consequences of the elimination of wolves from the vicinity of Yellowstone National Park. After the extirpation of wolves, elk proliferated and their subsequent overgrazing caused changes in the structure of the plant community, with cascading effects on other components of the ecosystem.

There are now efforts to restore ecosystems by reintroducing apex predators. The expectation is that the clock of change will run in reverse and former ecosystems will be restored. In the case of the wolf of Yellowstone, the return of wolves appears to be reversing some of the ecosystem change that resulted from their extirpation (Ripple and Beschta 2012). However, some argue that the ecosystem is not simply returning to its former state (Marris 2014). Perhaps we should not expect this to happen. One reason not cited by Marris for the failure of reversal is that the protagonists in the altered ecosystems may have evolved during the time when wolves were absent. In the absence of wolves, elk change the structure of the plant community as they deplete it of preferred species, then other ecosystem changes follow. Guppies, and other species living with guppies, like *Rivulus*, have evolved in a number of generations comparable to those experienced by elk and other species in the absence of predators. We should be considering how some of these species may have evolved during the absence of wolves and how this evolution might play a role in the change that occurs when wolves are reintroduced. If significant evolution has occurred, then a simple reversion to a former state will be unlikely. We need to instead adopt a different perspective, which is that change moves forward from the present state and is shaped by the present components of the community, which are not the same as they were in the past. The consequence is that we will only be able to predict the future if we are able to include evolution and feedbacks between ecology and evolution in our equations. Knowing the past is not sufficient to predict the future.

Pimentel's "genetic feedback" was well grounded in observations of natural systems, simulation models, and laboratory experiments, yet it remained dormant even as evolutionary ecology blossomed. I wonder why and suspect that one reason is that he hinged his arguments on evolution being rapid enough to keep pace with ecological interactions. Evolution was not popularly perceived this way in the 1960's. The perception of evolution as a contemporary process began with the birth of ecological genetics in the 1930's, because a central goal of the discipline was to integrate phenotypic variation with an assessment of the underlying genetic cause and the effects of this variation on survival and reproductive success (Ford 1971). However, the popular perception of evolution as a contemporary process did not begin to emerge until the 1980's, with the advent of well-documented examples, such as the Grants' work on *Geospiza fortis* (Boag and Grant 1981; Price et al. 1984; Grant and Grant 1989; Grant and Grant 1993; Grant and Grant 1995; Grant and Grant 2002). I think there has been an unheralded paradigm shift in

evolutionary biology because contemporary evolution was considered improbable by most in the 1960's, but is seen as routine today. With that shift, Pimentel's ideas became more palatable.

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