Eco-Evolutionary Feedbacks Predict the Time Course of Rapid Life-History Evolution

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ABSTRACT: Organisms can change their environment and in doing so change the selection they experience and how they evolve. Population density is one potential mediator of such interactions because high population densities can impact the ecosystem and reduce resource availability. At present, such interactions are best known from theory and laboratory experiments. Here we quantify the importance of such interactions in nature by transplanting guppies from a stream where they cooccur with predators into tributaries that previously lacked both guppies and predators. If guppies evolve solely because of the immediate reduction in mortality rate, the strength of selection and rate of evolution should be greatest at the outset and then decline as the population adapts to its new environment. If indirect effects caused by the increase in guppy population density in the absence of predation prevail, then there should be a lag in guppy evolution because time is required for them to modify their environment. The duration of this lag is predicted to be associated with the environmental modification caused by guppies. We observed a lag in life-history evolution associated with increases in population density and altered ecology. How guppies evolved matched predictions derived from evolutionary theory that incorporates such density effects.

Keywords: density-dependent evolution, density-dependent selection, eco-evo interaction, *Poecilia reticulata*, life-history evolution, experimental evolution.

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Introduction

The study of density-dependent selection represents a nexus of ecology and evolution (Travis et al. 2013). Theory from MacArthur (1962) through Engen and Sæther (2016) has explored how density-dependent selection differs from density-independent selection in its consequences and evolutionary dynamics. For example, when changes in population density feed back onto the expression of life-history traits, density-dependent selection can mold a life history different from what would evolve under density-independent selection (Charlesworth 1994; Engen and Sæther 2016). However, to make the problem tractable, most theory assumes that selection is weak, which allows the feedback from population density to the dynamics of alleles to be largely independent in time from the feedback from changing allele frequencies to population density (Otto and Day 2007). While this assumption makes the theory tractable, it can divert attention from the consequences of strong selection and the joint dynamics of population size and genes.

Pimentel (1961) explored these consequences of density-dependent evolution in an article entitled "Animal Population Regulation by the Genetic Feedback Mechanism": "Density influences selection; selection influences genetic make-up; and in turn, genetic make-up influences density" (p. 65). Said differently, Pimentel suggests that an increase in the abundance of an organism can cause changes to its ecosystem that can in turn impose selection on the organism, causing it to evolve. A possible consequence of such evolution is the ability to attain a higher population density, which can cause a new turn of the cycle between evolution and ecology.

Subsequent theoretical work has reinforced the logic of Pimentel's argument and demonstrated the potential importance of the way that density-dependent selection can mediate

a feedback between ecology and evolution (Levin 1972; Engen and Sæther 2016). The feedbacks in density-dependent selection are important not just for evolutionary dynamics but also for ecological dynamics. When numerical and genetic dynamics occur on the same timescale, a population or set of interacting populations can attain steady-state densities and relative abundances that are not predicted by ecological models without contemporaneous genetic dynamics (Roughgarden 1976). In particular, the inclusion of rapid genetic dynamics can promote species coexistence by stabilizing interactions that would otherwise be unstable (Abrams and Matsuda 1997).

The joint dynamics of numbers and genes can shape nature in profound ways (Travis et al. 2014*b*). For example, when a species invades a new habitat, its expanding population can alter its environment in ways that fulfill Pimentel's genetic feedback mechanism. The organism's impact on its ecosystem can shape its evolution in ways that affect further population growth; its impact on ecology may change, which could further alter the selection it experiences. The outcome of these postinvasion processes would be a feedback loop between the evolution of the target organism, accompanying evolution of other community members and changes in community and ecosystem structure (Antonovics 1992, 2003). Feedbacks between ecology and evolution are best known from the effects of contemporary evolution on the coupled population cycles of predators and prey, hosts and parasitoids, or hosts and pathogens (Pimentel 1961; Yoshida et al. 2003; Duffy et al. 2012). Here we present the first experimental demonstration in nature of a different facet of such feedback or how, as populations grow from low to high population density, they change their environment and by doing so shape their own evolution.

We are characterizing the dynamic feedback between ecology and evolution with experiments performed on guppies (*Poecilia reticulata*) in natural streams in the Northern Range Mountains of Trinidad. Guppies occupy rivers divided into discrete communities separated by waterfalls (Endler 1978). They live with multiple species of predators in the downstream communities. Waterfalls exclude most predators but not guppies from the higher reaches of streams and thus create two distinctly different communities in which guppies have evolved very different life histories (Reznick and Bryga 1996). Guppies from high-predation (HP) environments are younger at maturity, reproduce more often, devote more resources to each litter of young, and produce more offspring per litter compared to guppies from low-predation (LP) environments (Reznick and Bryga 1996). These are genetic differences that evolved independently in multiple drainages across the Northern Range Mountains (Reznick 1982*b*; Reznick and Endler 1982). Guppies transplanted from HP sites below waterfalls into predator- and guppy-free sites above waterfalls rapidly evolve life histories that match those of guppies found in natural LP communities (Reznick and Bryga 1987; Reznick et al. 1990, 1997). This response suggests that change in predator-induced mortality drives life-history evolution, as predicted in models of life-history evolution (Charlesworth 1994).

Differences among communities in predation are confounded with differences in ecology. Guppy populations found above barrier waterfalls, in communities with reduced predation risk (LP communities) attain far higher population densities than those from HP communities (Reznick et al. 2001). Guppies from HP environments have low population densities, higher somatic growth rates (Reznick et al. 2001; Reznick and Bryant 2007), and populations dominated by small, young individuals, a consequence of their high birth and death rates. In LP environments, there is instead a more even size distribution, with proportionately few small, young fish and more large, old fish (Rodd and Reznick 1997; Reznick et al. 2001). These differences in size distribution are a major contributor to the higher biomass density of guppies in LP environments. The juxtaposition of higher population densities and lower individual growth rates in LP guppies suggests an indirect effect of predators; in the absence of predation, guppies proliferate and attain high population densities.

This confounding of reduced risk of predation with increased population density begs the question, "Why do guppies evolve later maturation at a larger size in LP environments?" (Reznick and Endler 1982; Reznick and Bryga 1987). The ecology of guppy populations points to two hypotheses. First, the reduction in predation risk could elevate the survival rate of adults. This outcome would be a direct effect of the absence of predators. Second, an indirect consequence of reduced mortality risk is increased population density, which in turn imposed density-dependent selection on guppies.

A peculiar feature of these alternative hypotheses is that they can predict the same evolutionary outcome. Density-independent theory predicts that an increase in adult mortality risk will favor the evolution of earlier maturity and increased allocation to reproduction (Charlesworth 1994). This prediction appears to apply to guppies because some predators preferentially prey on large, adult size classes of guppies (Haskins et al. 1961; Seghers 1973). In an earlier study, we evaluated age- or size-specific survival with mark-recapture studies on seven HP and seven LP populations in three different rivers. Guppies from HP environments did indeed sustain higher mortality rates, but the increase in mortality risk was evenly distributed across all size classes (Reznick et al. 1996*a*). This result is problematic because the same body of theory predicts that life histories will not evolve if all age classes experience the same change in mortality rate; heterogeneity among age classes in mortality risk is necessary to drive life-history evolution (Charlesworth 1994).

Density-dependent selection theory can yield predictions consistent with the differences in life histories that we see between HP and LP environments even without a difference in age- or size-specific mortality. They can do so if density

regulation is attained through lower fecundity and/or higher juvenile mortality (Charlesworth 1994). We have since performed density manipulation experiments in natural LP communities and have demonstrated that guppy populations in LP environments are indeed regulated at ambient densities (Reznick et al. 2012; Bassar et al. 2013). Density regulation is attained via reduced fecundity and/or higher juvenile mortality, rather than via increased adult mortality (Bassar et al. 2013; J. Travis, personal communication), which is the demographic pattern of regulation predicted to cause the patterns of evolution we observe in LP environments (Charlesworth 1994). We have also demonstrated that guppies from LP environments are less sensitive to high population densities in short-term mesocosm experiments (Bassar et al. 2013). We thus have circumstantial evidence that it is the indirect effects of predators on guppy population densities, rather than the direct effect of predators on guppy mortality rates, that shape the evolution of the LP phenotype.

Hypotheses and Predictions

While the life-history phenotypes of the guppies by themselves cannot discriminate between these alternative hypotheses, the time course of evolution can. The experimental introduction of guppies from an HP environment to LP tributaries that did not already contain guppies instigates an episode of directional selection because guppies are transplanted from an environment where they experience high mortality rates to one where their mortality rates are substantially reduced (Reznick et al. 1996*a*). If this immediate change in mortality rate alone governs how guppies evolve, then evolutionary theory predicts that selection should commence immediately on introduction. Moreover, the strength of selection and rate of evolution should be highest at the outset and then decline as populations evolve toward the local optimum (Bulmer 1980; see the appendix; fig. 1; supplemental material, sec. I, figs. S1–S4; figs. S1–S9 are available online). If there is sufficient genetic variation to respond to the immediate onset of selection, then the life history will begin evolving in the initial generations. Alternatively, if the advent of density regulation followed by density-dependent evolution plays an important role in shaping local adaptation, then evolution will be delayed until guppy populations grow to the point that they exert a measurable impact on their ecosystem, which will initiate the density-dependent selection that they experience in this novel environment (fig. 1). Presuming that there is sufficient genetic variation to respond to selection, evolution will commence and will be correlated with population density.

Direct and indirect effects are not exclusive alternative hypotheses. It is possible for both to shape life-history evolution. If both were acting, the contribution of direct effects would decelerate over time while those of indirect effects would accelerate as density regulation comes into play. While the exact nature of their combined effects would depend on their relative strengths, we should see an initially high rate of evolution from the direct effects. The advent of indirect effects will sustain evolution beyond what is expected from direct effects alone.

There are other mechanisms that could also produce an apparent lag in the evolutionary response to an immediate onset of selection. We will address these alternatives in "Discussion" and show that when integrated with the trajectories of population growth, evidence for the advent of density regulation, and trends in heritability of male age and size at maturity, we can discriminate the two hypotheses illustrated in figure 1. We use the evolution of male age and size at maturation as our measure of local adaptation because theory predicts the evolution of delayed maturity under such circumstances (Charlesworth 1994; Engen and Sæther 2016). In addition, male guppies from LP environments are consistently older and larger at maturity than their counterparts from HP environments (Reznick 1982*b*; Reznick and Endler 1982; Reznick and Bryga 1996; Reznick et al. 1996*b*). Furthermore, when guppies were transplanted from HP environments into previously guppy-free LP environments, males rapidly evolved later ages and larger sizes at maturity; females evolved delayed maturity at a larger size at maturity as well, but it took at least three more years for female responses to be evident (Reznick and Bryga 1987; Reznick et al. 1990, 1997). These prior introduction studies demonstrated that the life history will evolve but could not tell us why it evolved.

Here we report on experiments in which we transplanted guppies from one HP locality into four guppy-free streams that previously contained the single fish species (*Rivulus hartii*) that naturally co-occurs with guppies in LP environments. We followed the guppy introduction with monthly mark-recapture censuses, yielding real-time characterization of population growth and the advent of density regulation. We pair these data with annual common-garden assessments of male age and size at maturity in which we compare male age and size at maturity in second-generation (G2) descendants from our four experimental streams with G2 descendants from the ancestral locality to make inferences about evolution in the introduced populations. We developed a pedigree for one of the four experimental streams for the first 5 years of the experiment or approximately 10–15 generations. The pedigree enables us to quantify lifetime reproductive success (LRS), additive genetic variance, and how these quantities change over time. The pairing of field and laboratory data enables us to evaluate the alignment between guppy life-history evolution and population dynamics and hence make inferences about the importance of density and density-dependent selection as a driver of life-history evolution. The genetic information enables us to discriminate among alternative explanations for our results.

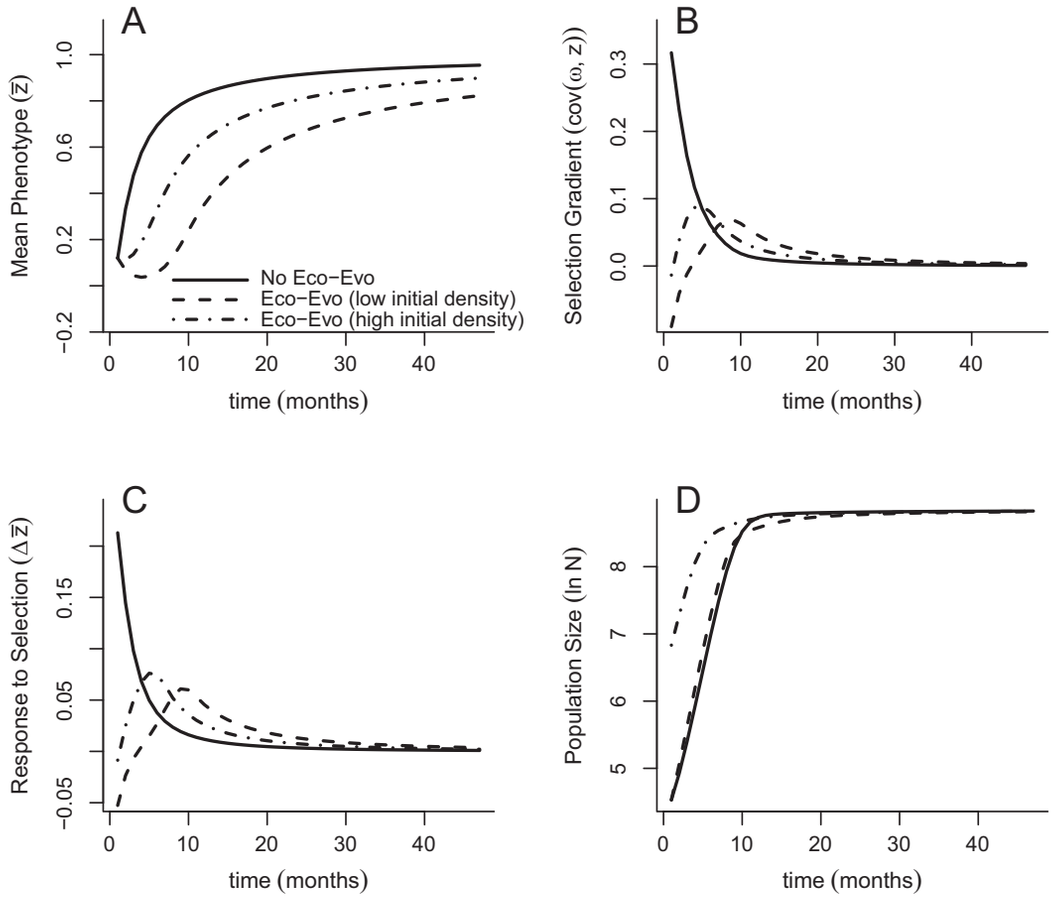

Figure 1: Temporal pattern of changes in the mean phenotype (*A*), selection gradient (*B*), response to selection (*C*), and natural log of population size (*D*) predicted by the model of density-independent selection (solid line; no eco-evo) and models of density-dependent selection (dashed lines; eco-evo). Selection is strongest in the density-independent model immediately after the population is established; in the density-dependent model, selection is weak at the outset and gathers strength as the population increases in density. When the initial density is very low in the density-dependent model (dashed line in *A*), selection can initially move the mean trait value in the opposite direction to the one that it will ultimately take.

We preceded and followed the introduction with assessments of the population biology of the other species of fish and the quantification of features of the ecosystem, including invertebrate and algae abundance. Those results either have been or will be published elsewhere and will be integrated with our interpretation of the results reported here. Our five sources of data (mark recapture, lab common garden, pedigree, characterization of the ecosystem, experiments in artificial streams) are summarized in box 1.

Methods

These new experiments differ from the earlier ones in five critical ways. First, they are replicated. Second, in the earlier introductions we waited 4 years before beginning to look for evolution in the introduced fish and did not collect data on the population dynamics of the introduced populations. Here we began assaying for the evolution of male age and size at maturity after 1 year and did so every year for 4 years. We coupled these assays with the quantification of the population dynamics in the experimental streams. Third, in the former experiments we introduced mixed size/age groups of fish transplanted directly from the ancestral to the experimental site. In the current study, we instead introduced fish that were collected as juveniles, reared to maturity in single-sex groups, and then mated and individually marked before introduction. This difference means that in the current study we know all founders. In prior experiments we did not because adult females carried stored sperm. Fourth, we tracked the population dynamics of the introduced populations with

Box 1: Sources of data and the dependent variables defined by each source

1. *Lab common garden*. Estimates of age and size at maturity in the second laboratory-born generation of males from the ancestral and introduction sites. Performed once per year in years 1–5 for the first pair of streams and years 2–5 for the second pair, initiated a year after the first pair.
2. *Monthly censuses of introduction sites*. These provide estimates of population density, mortality rate, and individual growth rates.
3. *Pedigree (available for only one of the four introduction sites)*. These data are combined with monthly censuses to yield estimates of individual reproductive success and heritability of male size at maturity.
4. *Before/after, control, and introduction data on the ecosystem and killifish mark recapture*. One year before the guppy introductions, we initiated monthly assessments of invertebrate abundance and aufwuchs and bimonthly mark-recapture of killifish in the introduction sites and a control region upstream of the introduction site, above a natural barrier that excludes the introduced guppies. These results, presented in other publications, are cited where appropriate in the current article.
5. *Artificial stream experiments*. In parallel with the introductions, we conducted a series of factorial experiments in 16 artificial streams in which we compared the performance of guppies derived from natural HP and LP environments. Our goal was to characterize what the two end points of the process of adapting to these alternative environments is like when compared in a common environment. These results define, among other things, how each phenotype responds to increases in population density. These results, presented in other publications, are cited where appropriate in the current article.

high-resolution monthly mark-recapture censuses and characterized their impact on the ecosystem. Fifth, we kept scales from all founders and all recruits to serve as a source of DNA for construction of a pedigree and estimation of individual reproductive success. The pedigree data are currently available for only one of the four replicates because of funding limitations.

We quantified the evolution of the transplanted guppies with common-garden laboratory studies performed once per year on the second generation of laboratory-born fish descended from wild-caught juveniles from the four introduction sites and the ancestral site, which serves as the control. We inferred evolutionary change from differences in trait values between the ancestral and the experimental populations as expressed in guppies raised in a common developmental environment that was two generations removed from nature.

Initiation and Sampling of the Experimental Populations

Four experimental populations of guppies were established as paired introductions in 2008 and 2009 in the upper Guanapo River drainage in the Northern Range Mountains of Trinidad, West Indies. We translocated guppies from a single HP source population in the lower Guanapo River into four upstream reaches, with two in each year. Natural and enhanced barriers bound each reach and prevent immigration of native guppies into the experimental reaches and emigration of the introduced guppies into the control regions above the introduction sites. The experimental reaches and stretches of stream downstream from the experimental reaches previously lacked natural guppy populations but otherwise had habitat attributes similar to other LP streams in Trinidad.

We collected the introduced fish as juveniles, reared them to maturity in single-sex groups, and then mated them in groups of five males and five females. We introduced them into the experimental streams before the females produced their first litter of young. We did so in March, the beginning of the dry season, when the rate of reproduction and population growth is highest. We introduced 38 pairs of fish into each of the first two streams (Upper Lalaja [UL] and Lower Lalaja [LL]) in 2008 and 52 pairs of fish into each of the second pair of streams (Taylor [TY] and Caigual [CA]) in 2009. An additional 12 pairs were introduced into CA within days of the first introduction, bringing the total for that stream to 64 pairs. When we introduced the 38 and 52 pairs of guppies, we placed the females from a breeding group into one stream and the males into the other, with the consequence that the male contribution to both streams was the same. Half of the males were represented as live fish and the other half as products of mating before release, including developing embryos and stored sperm. We individually marked all fish, beginning with the founders, with subcutaneous injections of colorized elastomer (Northwest Marine Technologies). We had eight possible places to apply marks and 12 different colors of elastomer, and we applied two marks per fish. The resulting combination of positions and colors enabled us to uniquely mark 4,032 individuals of each sex. We censused all populations once per month and had an average of >90% probability of

seeing an individual if it was alive at the time of the census (table 1). We photographed and weighed all fish each time they were caught. These data enabled us to estimate population size, size structure, population density, biomass density (grams of guppy per square meter of stream), and individual growth rates for each census interval.

We collected fish with aquarium or butterfly nets while standing on the shore or rocks so as not to disturb stream substrate. Males and females were stored in separate Nalgene bottles that were labeled for site of origin, kept separately throughout subsequent processing, and then returned to the site of capture. All fish were transported to our laboratory and kept in aerated aquaria until processed. They were lightly anesthetized for processing, identified by mark, sexed, weighed, photographed for later length measurement, and then returned to their holding tank. All unmarked fish ≥14 mm standard length (SL; new recruits) were uniquely marked. The processed fish were kept overnight in medicated water and then returned to the site of capture. We did not mark and measure smaller fish because doing so results in some mortality.

Sampling of Guppies for Laboratory Common-Garden Experiments

The HP source population (hereafter, "source") was collected from a downstream site where guppies coexist with a suite of predator species, including the pike cichlid (*Crenicichla* spp.), a major predator on guppies (Gilliam et al. 1993; Torres Dowdall et al. 2012). Forty to fifty juveniles were collected from the first pair of introduction sites (UL and LL) in 2009 (three or four generations after being introduced), 2010 (six to eight generations), 2011 (nine to 12 generations), and 2012 (12–16 generations). Forty to fifty wild-caught juveniles were sampled from the second pair of introduction sites (TY and CA) in 2010 (three or four generations after being introduced), 2011 (six to eight generations), and 2012 (nine to 12 generations). Forty to fifty juvenile guppies were collected from the source population at the same time in all 4 years. We used these wild-caught juveniles to initiate our laboratory populations. In natural HP and LP environments, juveniles have only a 10%–20% chance of attaining adulthood (Reznick et al. 1996*a*), so using juveniles rather than adults to initiate our lab stocks minimizes our impact on the experimental populations. We always collected only a few juveniles per pool and sampled evenly from the full length of the experimental reach when assembling our populations for export. The intent was to randomly sample the full genetic diversity of stream and—because juveniles tend to remain in the pool where they are born until they are much larger—to avoid biasing the sample by including multiple siblings from a single litter. All collections on all years were made by D. N. Reznick, assuring that the sampling process was uniform.

Table 1: Mean survival and probability of capture for first 48 months of experimental introductions

Parameter and sex	Estimate	SE	LCL	UCL
Survival:				
Female	.870	.0012	.8678	.8725
Male	.735	.0023	.7303	.7393
Probability of capture:				
Female	.904	.0012	.9013	.9061
Male	.922	.0019	.9187	.9261

Note: Estimates and SEs are from a Cormack-Jolly-Seber survival model implemented in program MARK. The estimated probabilities of survival in our earlier comparisons among low- and high-predation localities were 54% and 25% for males and 78% and 57% for adult females, respectively, for a time interval of 30 days. The numbers in the table pertain to survival for a time interval of 30 days. LCL = lower 95% confidence limit; UCL = upper 95% confidence limit.

Common-Garden Rearing Protocol

To minimize maternal and other environmental effects on our dependent variables, we reared all wild-caught guppies for two generations in custom-made recirculating systems under common-garden lab conditions as described in Torres-Dowdall et al. (2012), Handelsman et al. (2013), and Ruell et al. (2013). We reared the wild-caught juveniles to maturity in single-sex groups. We randomly outcrossed females with males from different families to produce first-generation (G1) laboratory-born individuals. G1 individuals were reared to maturity and randomly outcrossed to produce full-sibling broods of G2 laboratory-born individuals.

We reared full-siblings in two 1.5-L tanks (two to 10 full siblings per tank) at high and low food levels. The tanks were part of a flow-through rack system with continuous filtration and ultraviolet sterilization. We fed guppies in the high food treatment quantified rations that approached ad lib. (a.m.: Tetramin tropical fish flakes, Spectrum Brands, Cincinnati, OH; p.m.: brine shrimp nauplii, *Artemia* spp.) and were comparable to high food levels administered in earlier experiments (Reznick 1982*b*). We fed guppies in the low food treatment half the daily food allotments of guppies in the high food treatment. In both food treatments, food levels were adjusted weekly for age and number of individuals per tank. We included food level in initial analyses but removed it from those reported here because there were never any interactions between food and other factors in the model. Not including food simplifies the presentation.

At 29 days old, we anesthetized G2 juveniles in tricaine methanesulfonate (MS-222; Sigma-Aldrich, Saint Louis) and sexed them. Juvenile males can be differentiated from females based on the presence or absence of melanophores in a triangular patch that appears on their ventral abdomen. This patch is present only in females (Reznick 1982*b*). Once sexed, we individually housed one to five males per family

per rearing treatment and reared them under constant conditions until they reached sexual maturity.

Age and Size at Maturity

Males are considered to be sexually mature when the apical hood grows even with the tip of their gonopodium (Reznick 1982*b*, 1990). We checked males weekly for the first appearance of the apical hood. Subsequently, we checked them daily until the day they reached maturity. We then anesthetized them, spread them laterally along a white background alongside a metric ruler, and digitally photographed them with either a Panasonic DMC FZ8 digital camera (Panasonic, Secaucus, NJ) or a Canon EOS Rebel XSi SLR digital camera (Canon, Melville, NY). We measured SLs from photographs using ImageJ version 1.44 (Abràmoff et al. 2004).

Pedigree Construction

We implemented microsatellite analysis (43 loci with an average of 12.25 alleles per locus in founders and 9.90 alleles per locus at the end of December 2011) to reconstruct the pedigrees of one of the experimental populations (LL) for the years 2008–2013. Our available funds have limited us to developing a pedigree for only one of the four populations thus far. The pedigree includes the founders and all recruits because we collected scales from the founders before releasing them and then collected scales from every new recruit as part of our monthly censuses. We amplified microsatellite genotypes in large multiplexes, and then genotypes were scored with MEGASAT (Zhan et al. 2017). We reconstructed the pedigree using the program COLONY (Jones and Wang 2010), which accounts for the likelihood of both parental and sibship relationships, thus using information on entire clusters of relatives to reconstruct parent-offspring pairs. Our estimated monthly probabilities of capture (if alive) are high, and the probability that a given individual reaches maturity and dies uncaught is <0.01, thus minimizing the proportion of missing parents. We used the pedigree to quantify LRS as the number of offspring recruited into the marked population, which means they attained an SL of at least 14 mm. We quantified LRS for the founding males and all males born through December 2011. The end dates were chosen to include cohorts for which we had complete LRS for all members of the cohort. Males rarely live for more than 4 months after maturity, but their sperm can remain viable in inseminated females for months after they die (Lopez-Sepulcre et al. 2013). The 2-year gap between 2011 and 2013 means that all males born in 2011 had been dead long enough for all offspring that could have been sired by their stored sperm to grow large enough to be included in the mark-recapture study. Since there are two or three generations per year, this estimate applies to the first eight to 12 generations.

Estimating the Opportunity for Selection

We estimated the opportunity for selection (I), which is the variance in relative fitness (Crow 1958), from the data on LRS pedigree. We estimated I as the variance in male LRS divided by the square of mean male LRS (Arnold and Wade 1984). This index acts as an upper limit on the intensity of selection; $I^{1/2}$ is the maximum number of phenotypic standard deviations that any character under selection can shift in a single generation of selection. While I does not measure the actual intensity of selection on a character, it is a useful comparative measure that indicates the circumstances under which selection can be stronger or weaker. In particular, low values of I indicate circumstances under which selection must be weak. We use I to characterize how the opportunity for selection changed between the initiation of the experiment and the end of 2011.

Data Analysis for the Common-Garden Experiments

We tested for evolutionary divergence and phenotypic plasticity in two male life-history traits (age at maturity [days] and size at maturity [mm SL]) with Bayesian generalized linear mixed effects models (GLMMs) in the R package MCMCglmm (Hadfield 2010; Hadfield and Nakagawa 2010). We used this approach instead of a standard ANOVA because it allowed us to incorporate information on the relatedness of the individuals in the experiment using a pedigree. We analyzed each pair of introduction streams (UL/LL and TY/CA) separately because they span different years. For the linear model for each pair of streams, we included year, stream, food level, and their interactions as fixed categorical effects. The family identity of full siblings was modeled as a random effect. For the fixed effects, we used a means parameterization model structure. This means that posterior distributions for each of the parameters in the model corresponded to the posterior distribution of the means of each of the streams and food levels in each year. GLMMs were run for 1,300,000 iterations with a thinning interval of 100 and a burn-in period of 300,000 iterations to estimate the posterior distribution and minimize autocorrelation. The Markov chain was sampled 10,000 times to estimate the variances of the fixed and random effects. Parameter-expanded priors were used in all models (Hadfield 2010). Plots of all posterior distributions were visually inspected to confirm that each model properly converged and for autocorrelation. We also calculated autocorrelation and found it to be less than 0.06 in all models. Plots of all variances were visually inspected and approximated normal distributions.

We then calculated the main effects of introduction and food treatments and their interaction for each year by constructing linear combinations from the posterior distribution. The structure of the linear combinations corresponded to

what would typically be done in a factorial ANOVA. For example, for each year, the evolution effect was calculated as the difference between the mean of the two introduction streams across food treatments minus the mean of the control values across food treatments. These contrasts were calculated from each set of sampled parameter distributions from the Markov chain to yield a contrast distribution for each effect. All analyses were performed in R version 3.2 (R Development Core Team 2014).

Sample sizes appear in table S3 (tables S1–S9 are available online). Details of the results of the statistical analyses of male age and size at maturity, reported separately for each pair of streams, are reported as tables S4–S7.

Integration of Common Garden and Biomass Density in the Introductions

We assessed the role of density dependence in the introduced populations by relating the divergence of the introduced populations from the source population to the cumulative biomass density in the year prior to the capture of the fish used in the common-garden experiment. We did so with a general linear model approach to ANCOVA. The divergence between the ancestral and introduced populations was estimated in the laboratory common-garden experiments. The biomass density was estimated from the monthly censuses. Cumulative density in the year prior was calculated as the sum of the biomass of guppies in each stream from the February census in each year to the March census in the prior year divided by the estimated benthic area of each stream. The benthic area of each stream was calculated using morphological measurements of 50 m of each stream and multiplying this measure by the overall length of the stream. We treated cumulative density as a continuous predictor and stream as a fixed categorical effect. We initially included an interaction between stream and cumulative biomass density but removed this interaction as it was not statistically significant. The cumulative densities were intended to be representative of the strength of density-dependent selection experienced by the grandparents and great-grandparents of the guppies in common garden. The grandparents were the wild-caught juveniles used to initiate the laboratory common garden, so our density assessment includes the density that they and their parents experienced in the natural environment. Significance values for the relationship between age and size at maturity and cumulative biomass density were calculated using the nonparametric bootstrap. For each bootstrap replicate, we subsampled data at random with replacement. We used 20,000 bootstrap replicates, which was more than sufficient to ensure that the bootstrap distributions converged. Bootstrap *P* values were calculated as the proportion of analyses with coefficients less than zero so that the reported *P* value is one sided.

Results

Mortality, Growth Rate, and Lifetime Reproductive Success

The probability of survival in the introduction sites was higher than in natural LP and HP sites (Reznick et al. 1996*a*; table 1; supplemental material, sec. II, figs. S5–S8, table S1). The transplant from an HP site to an LP site thus had the expected effect of increasing survivorship. A consequence of higher survivorship was that population density increased through the first 2–3 years of each introduction, punctuated by declines associated with the rainy season, when periodic floods scoured food resources out of the streams, which in turn caused a cessation of reproduction and recruitment (fig. 2).

The increase in population density affected individual performance. Juvenile growth rate declined progressively with time and increased density in all four populations (table 2; fig. 3). This decline most likely reflected reduced food availability. There were no systematic changes in water temperature, which might also have affected growth rate. At the same time, algae and invertebrate abundances, the two main sources of food, declined in the introduction sites relative to guppy-free control sites upstream, above barrier waterfalls that excluded the introduced guppies (Simon et al. 2017). Our common-garden experiments also revealed that juvenile growth rate had not evolved in a fashion consistent with this progressive decline in growth rate (supplemental material, sec. III, table S2, fig. S9).

The opportunity for selection increased progressively with the increase in population density. The average LRS, estimated as the number of offspring that are recruited from each male, declined precipitously as population density increased ($F_{1,41} = 10.63$, $P < .002$; fig. 4*A*). There is a positive outlier that represents an early data point, when the population density was low and LRS was exceptionally high. The relationship remains negative and significant ($P = .02$) when this point is removed. This decline demonstrated that fitness was density dependent. At the same time, the opportunity for selection, the standardized variance in fitness, increased with increases in population density ($F_{1,41} = 6.81$, $P = .012$; fig. 4*B*). There is again a positive outlier; the significance of the relationship is higher with that data point removed ($F_{1,40} = 8.24$, $P = .007$). We report these results for males because we are focused here on the evolution of male traits; results for females (not shown) are the same. This progressive increase in the variance in male reproductive success sets the stage for an evolutionary response at higher densities if some component of this fitness variation has a genetic basis.

Life-History Evolution

Our annual assessment of male age and size at maturity in common-garden conditions reveals that later age and larger

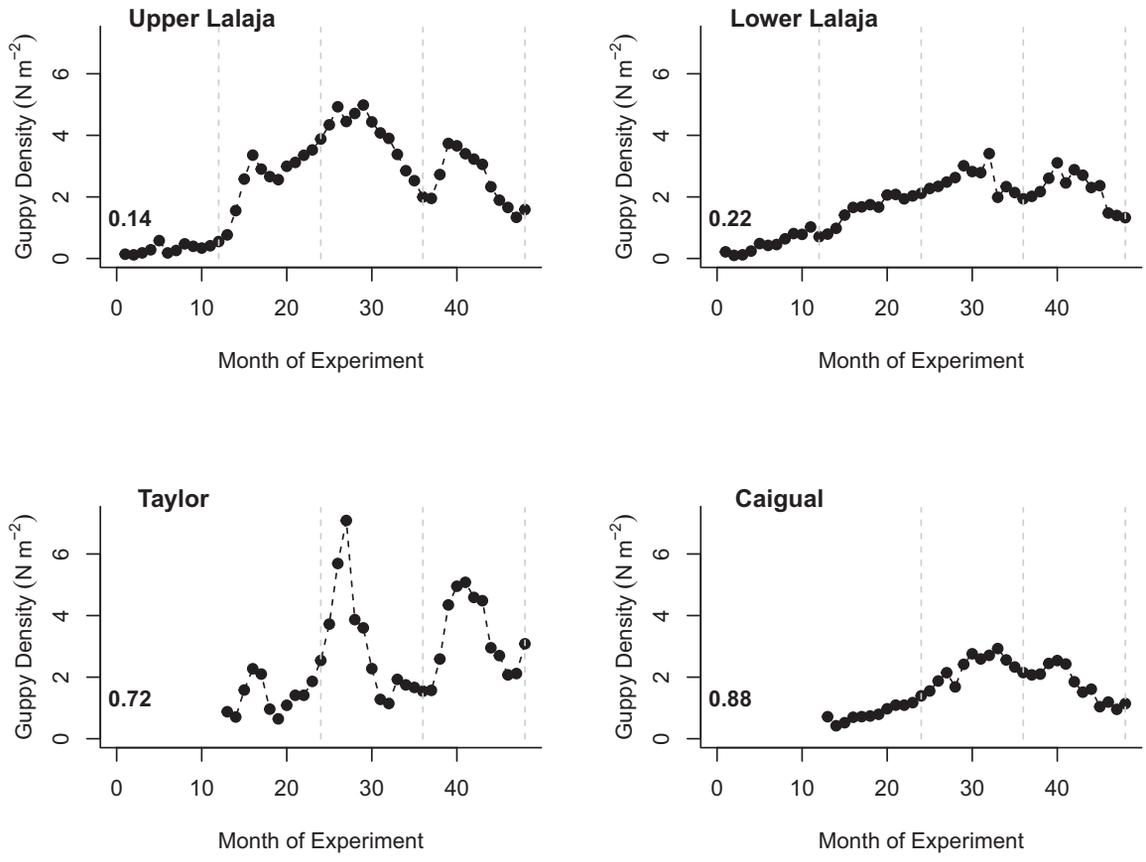

Figure 2: Total population densities in the four introduction streams since the first introduction. Population densities were corrected for capture probabilities specific to sex, stream, and census period by dividing the number of individuals caught by the probability of capture estimated using a Cormack-Jolly-Seber model implemented in program MARK. Guppies were introduced into Upper and Lower Lalaja in March 2008 and in Taylor and Caigual in March 2009. Vertical lines represent years since the introduction. Initial densities for each stream are located in the lower left-hand corner of each plot.

size at maturity evolved in all four streams but with a 2–3-year lag between the guppy introduction and evolution (fig. 5; supplemental material, sec. IV, tables S3–S7). This lag in evolution is consistent with the hypothesis that guppies were adapting (at least in part) to their increase in population density and consequent impact on the ecosystem, rather than to the reduced risk of mortality (fig. 1).

The two pairs of experimental streams differed in the duration of the lag (fig. 5). In the first pair of streams (UL and LL), there was a 3-year lag before later ages and larger sizes appeared (fig. 5). Males were older and larger at maturity in both years that followed. In contrast, the second pair of streams (TY and CA) had only a 2-year lag; males were older and larger in the second and third year, but the size differences fell short of statistical significance in the third year (fig. 5).

This difference between stream pairs was associated with differences in the initial population densities and the time required to attain peak density. The first pair of streams is larger, and the number of fish introduced was smaller (78 per stream in the first pair vs. 104 and 126 per stream in the second pair), resulting in three- to sixfold higher initial population densities in the second pair of streams (0.14 and 0.22 fish per square meter in the first pair vs. 0.72 and 0.88 fish per square meter in the second pair). Introducing more fish into the second pair of streams translated into their attaining peak population density 1 year earlier than in the first pair (fig. 2).

We further tested the hypothesis that the difference in lag time was caused by differences in density increase by comparing the results for age and size at maturity, estimated from our common-garden laboratory experiments, with the cumulative natural biomass density in the previous year for each year of the study. This is the density experienced in the field by the two prior generations of the wild-caught progenitors of our lab stocks. We found a positive correlation between density in each stream in the year before collection of the progenitors of the lab fish and difference in age and

Table 2: Somatic growth increment (mm SL month^{-1}) of female guppies in the four introduction streams

Stream	β_0	β_{SL}	β_N
Upper Lalaja	5.37 (.083)	−.41 (.007)	−2.10 (.034)
Lower Lalaja	4.16 (.109)	−.37 (.010)	−2.69 (.084)
Caigual	3.14 (.068)	−.22 (.005)	−.30 (.053)
Taylor	6.04 (.090)	−.39 (.007)	−.74 (.023)

Note: SL_t is the standard length at time t, measured as the distance from the tip of the snout to the hypural plate in the tail. The parameter estimates below are from the model $SL_{t+1} - SL_t = (\beta_0 + \beta_{SL}SL_t)e^{\beta_N N} + \varepsilon$, where $SL_{t+1} - SL_t$ is the change in SL from month t to month $t + 1$. The parameters β_0 and β_{SL} together give the density-independent growth increment assuming von Bertalanffy growth. The parameter β_N is the density-dependent parameter, and N is the total biomass density (g m^{-2}). When β_N is negative, the growth increment declines with increasing density. The estimated parameters in this table are the means and standard deviations. SL was centered on 14 mm prior to analysis so that the predicted relationship between growth and density is for a female guppy just recruiting into the size class that we census. The model was fitted using the bbmle package in R (Bolker and R Development Core Team 2014). All parameters were significant at the $P < .001$ level.

size at maturity between introduced and ancestral populations (fig. 6; P values for one-tailed tests for age-density and size-density correlations are 0.017 and 0.039, respectively). We applied one-tailed tests to these statistics because our prior experiments and comparative studies revealed that guppies consistently evolve later ages and larger sizes at maturity in LP environments (Reznick 1990; Reznick and Bryga 1996; Reznick et al. 2012; Bassar et al. 2013). These trends further support the argument that population density and the ecological changes associated with higher density, both attributable to the absence of predators, contributed to shaping the evolution of male age and size at maturity.

Discussion

Our results indicate that increased population density and the associated impact of guppies on their ecosystem were the primary causes of selection on male age and size at maturity. We inferred this connection between density, ecology, and selection from contrasts between the portions of stream where guppies were introduced and our upstream controls, from which guppies were excluded by waterfalls. In comparison with the controls, the portion of streams with introduced guppies were depleted of algae and invertebrates (Simon et al. 2017). The presence of guppies was also associated with a decline in killifish abundance (Fraser and Lamphere 2013). At the same time, there were progressive declines in the growth rates of juvenile guppies (table 1; fig. 3) and individual reproductive success (fig. 4*A*). The decline in reproductive success was accompanied by a substantial increase in the variance in reproductive success, which in turn caused an increased opportunity for selection in males and females (fig. 4*B*). All of these changes implicate the importance of increased population density and the impact of density on the ecosystem as drivers of guppy evolution.

The evolution of male age and size at maturity, inferred from the common-garden studies, happened only after the introduced populations attained peak densities and impacted their ecosystem. The duration of this lag differed in the two pairs of streams and did so in a way that was aligned with differences in the onset of evidence for density regulation (fig. 6). The first pair of streams was introduced at a much lower initial population density, took a year longer to attain peak densities, and also took a year longer to reveal evidence of life-history evolution. Together, the data on guppy population dynamics, changes in ecosystem structure, and evidence for a lag in life-history evolution indicate that age and size at maturity did not evolve solely as a direct consequence of the lower mortality rates associated with a reduced risk of predation (fig. 1).

This conclusion is consistent with earlier analyses of the evolution of offspring size in guppies. Guppies from LP environments produce larger offspring, there is a genetic basis to these size differences, and increased offspring size has evolved in the context of our longer-term introduction experiments (Reznick 1982*a*, 1982*b*; Reznick et al. 1990; Reznick and Bryga 1996). All available evidence argues that the evolution of larger offspring is an adaptation to restricted food availability rather than predation (Bashey 2002, 2008; Jorgensen et al. 2011).

This conclusion also aligns with our assessment of the relative fitness of guppies derived from natural HP and LP environments and then reared at high and low population densities in replicate artificial streams (box 1). Guppies from HP environments had substantially higher fitness than those from LP environments when reared at low population densities but lost this fitness advantage when reared at high population densities (Bassar et al. 2013). When our introduced populations attained peak densities, their population dynamics became consistent with the advent of density regulation. That regulation was attained by either reduced fecundity or recruitment, rather than through adult mortality (J. Travis, R. Bassar, T. Coulson, A. Lopez-Sepulcre, and D. Reznick, unpublished manuscript). This form of age-specific regulation represents the conditions under which life-history theory predicts the evolution of delayed maturation and reduced allocation to reproduction (Charlesworth 1994). We argue that the alignment of the new results reported here with prior experiments, our analyses of population regulation, and the way regulation is attained with life-history theory together make a strong argument for a dominant role of density in shaping how guppies adapt to LP environments.

Alternative Explanations

Our argument for density-dependent selection as a driver of evolution attributes the lag in evolution to a lag in the onset

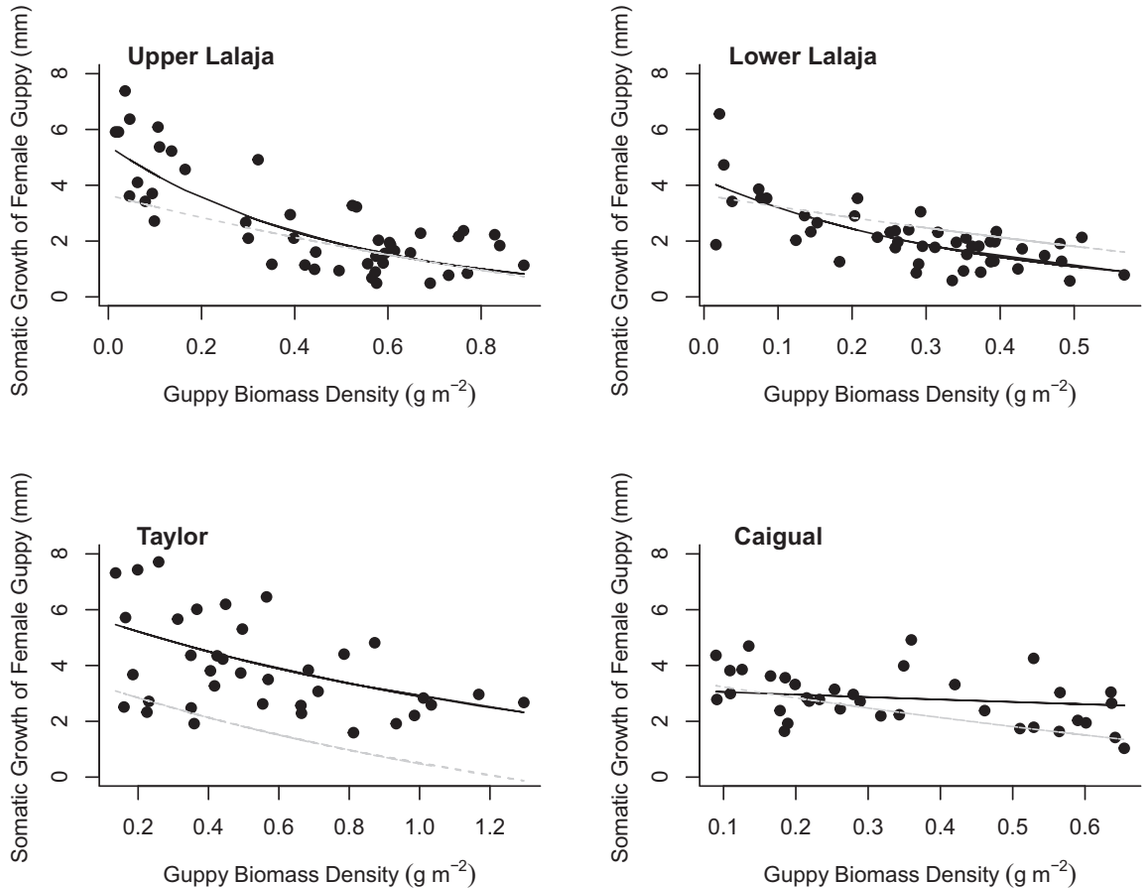

Figure 3: Somatic growth rate of 14-mm standard length (SL) female guppies from the focal streams as a function of biomass density. Solid black lines represent the estimated relationship between growth and total biomass densities in the four introduction streams. Growth for each stream was modeled as $SL_{t+1} - SL_t = (\beta_0 + \beta_{SL}SL_t)e^{\beta N} + \varepsilon$. The parameters β_0 and β_{SL} together give the density-independent growth increment. The parameter β_N is the density-dependent parameter describing how the growth increment changes as a function of biomass density, *N*. SL was centered on 14 mm prior to analysis so that the predicted relationship between growth and density is for a female guppy just recruiting into the censused population. Values of ε are normally distributed residual errors. Dashed gray lines are the relationship between growth of 14-mm female guppies and total biomass densities estimated using the same model from five natural populations of guppies that live without predators. Populations were manipulated by increasing, decreasing, or holding the densities constant in pool habitats over a 1-month period (data from Bassar et al. 2013).

of selection on life histories. We argue that selection for later age and larger size at maturity did not emerge until the populations had achieved high densities because of the effects that high guppy densities have on their ecosystem. This argument implies that upon introduction, the HP-derived fish were close to the optimal age and size at maturity for low population densities. As density increased, the optima shifted to later ages and larger sizes. This interpretation is consistent with results from mesocosm experiments that suggest that the advantage of the HP phenotype over the LP phenotype diminishes as population density increases (Bassar et al. 2013).

The alternative hypothesis for the delayed evolution we observed is that the reduced risk of adult mortality, or the existence of some other as yet unknown ecological agent unrelated to population density, imposed selection for later age and larger size at maturity immediately upon introduction of guppies. In this argument, the mean character values in the HP-derived fish were far from the optima in the new environment and the lag in response that we observed was caused by factors other than a lag in the onset of selection.

There are many factors that could have caused the lag, even if selection began immediately (summarized in table 3). These factors fall into three categories. First, selection might have been weak, either initially or consistently through time. Second, a variety of genetic constraints could have delayed the response to immediate selection, whether strong or weak. Third, maternal effects or phenotypic plasticity might have counteracted selection in the early generations.

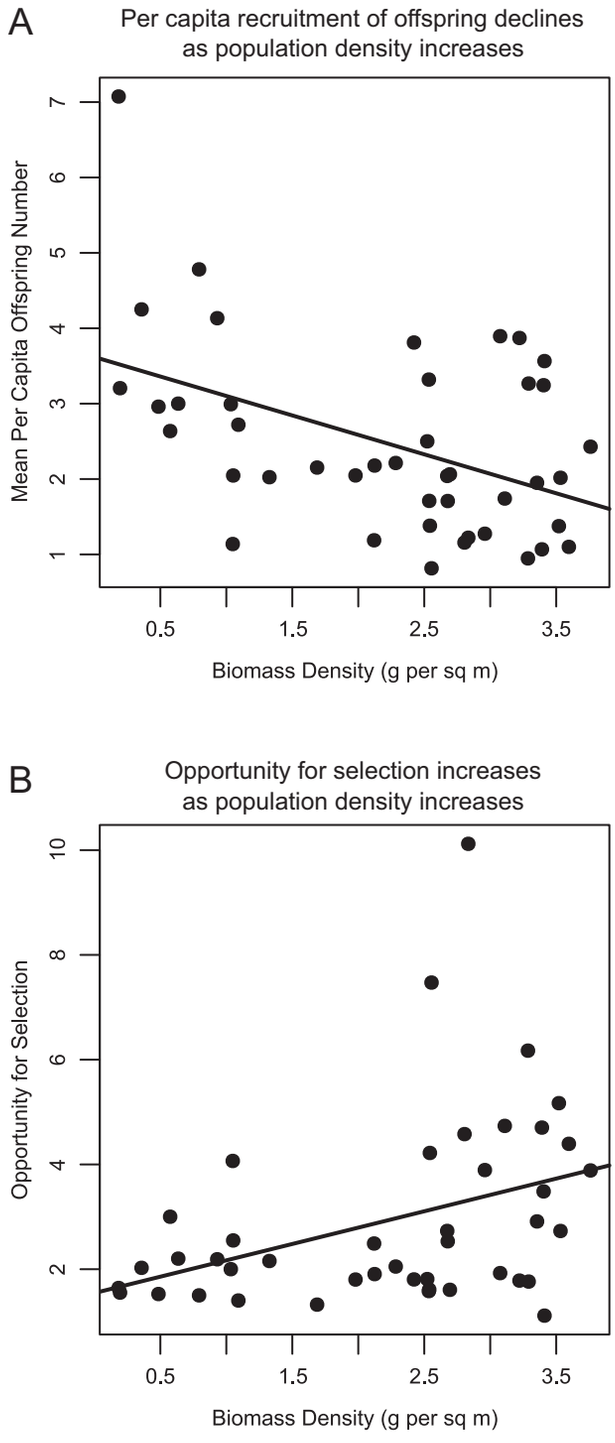

Figure 4: Mean number of offspring recruited per sire per month of the study declined with increasing population density. The pedigree for the Lower Lalaja river, where guppies were introduced in 2008, enables us to identify the mother and father of newly recruited offspring, meaning those large enough (>14 mm standard length) to be able to mark. This figure plots the average number recruited per male as a function of the population density for the first 45 months of the study (April 2008–December 2011). There was no recruitment for the first 2 months of the census, leaving us with 43 months of data on new recruits. We truncated the time period for which male reproductive success was quantified to include only monthly cohorts for which we had complete assessments of reproduction. Some members of cohorts born after this date may have had offspring recruited after the time interval represented by our pedigree. Because population density and time are well correlated, the figure with time as the *X*-axis is qualitatively similar to the ones pictured here. *B*, Opportunity for selection among males increased with increasing population density. The regression line in the figure is fitted to the raw data; the regression in the text was performed on the log of the opportunity to meet assumptions of linear regression. The opportunity is the variance in reproductive success standardized by the square of mean reproductive success. The opportunity for selection is a standardized estimator of the upper limit for any selection differentials. Data underlying this figure have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.2687ks0; Reznick et al. 2019).

Weak Initial Selection. There are two potential ways in which weak selection could have produced a lagged response. In the first, selection, though immediate, might have been initially weak, making the initial evolutionary responses too small to be detected. This could happen if the relationship between fitness and the trait value in the new environment resembled a normal distribution, with the optimum trait value at the peak of the curve, and if the initial trait values were not only far to the left of the optimum but far to the left of the inflection point of the curve. Were this the case, despite the immediate onset of selection and evolution, the initial response would be slow because of the low slope of the fitness surface at that point. The response would accelerate as the population mean approached the new optimum phenotype.

For this hypothesis to be true, both the variance in the initial trait distribution and the variance in the fitness function would have to have been very small (supplemental material, sec. I). A small variance in the fitness function, with a peak centered around the LP phenotype and a small phenotypic variance in the initial trait distribution, would imply that the mean absolute fitness would also have been very low, perhaps even below replacement level, causing very slow or even negative population growth (fig. S23). Instead, we saw immediate, rapid population growth in all four introduced populations, with adult numbers increasing at a rate of ~6% per month in three of them and almost 25% per month in the fourth (fig. 2). We illustrate this argument with a simple evolutionary model in section I of the supplemental material.

The second argument for an effect of weak selection is based on the relationship between the strength of selection and the effective population size. In this argument, stochastic effects associated with the small population sizes in the early stages of the experiment could have precluded an immediate response to selection. Only in later generations, when the population sizes were larger, would there have been any detectable response. The foundation of this idea is that unless the

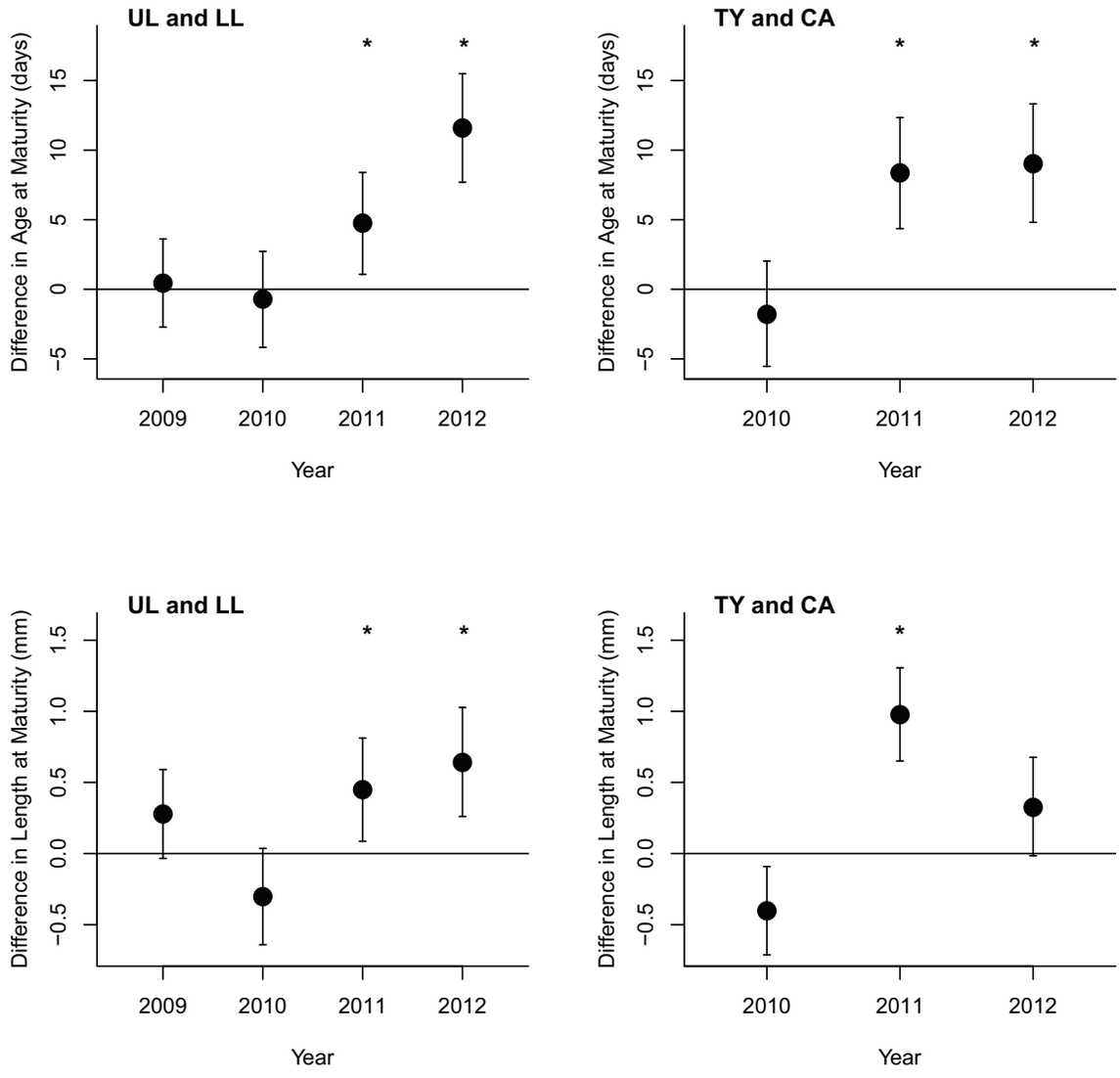

Figure 5: Differences between the age and size at maturity of male guppies from the introduction streams and the age and size at maturity of male guppies from the source population on the Guanapo River presented as a function of calendar year. Points and error bars represent the means and 90% credible intervals of the posterior distribution of the difference between the introduced and source populations. Asterisks denote significance for the one-tailed test. Our choice of a one-tailed test was based on our prior knowledge that transplantation from high-predation to low-predation environments causes the evolution of later age and larger size at maturity (Reznick et al. 1990, 1997). Data are from common-garden experiments performed on the grand-offspring of fish captured from the wild in each of the years listed on the *X*-axis. CA = Caigual; LL = Lower Lalaja; TY = Taylor; UL = Upper Lalaja. Data underlying this figure have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.2687ks0; Reznick et al. 2019).

selection coefficient associated with an allele exceeds $1/4N_e$, where N_e is the effective population size, drift will govern the dynamics of allele frequencies (Crow and Kimura 1970). While the requirements for selection to drive the dynamics of alleles in polygenic models are more complicated (Lynch 1984), it is still true that weak selection in a very small population is unlikely to be effective. Thus, had selection begun immediately on introduction and remained weak but constant, we might not have seen a response until the populations had exceeded a threshold size determined by the magnitude of selection.

Had this been the case, the response to selection would have been independent of density once the population sizes passed that threshold. In fact, the responses were proportional to density throughout the range of densities we encountered (fig. 6). Furthermore, there was a continuous increase in the opportunity for selection (I) as population density increased in LL, a pattern unlikely under this alternative scenario.

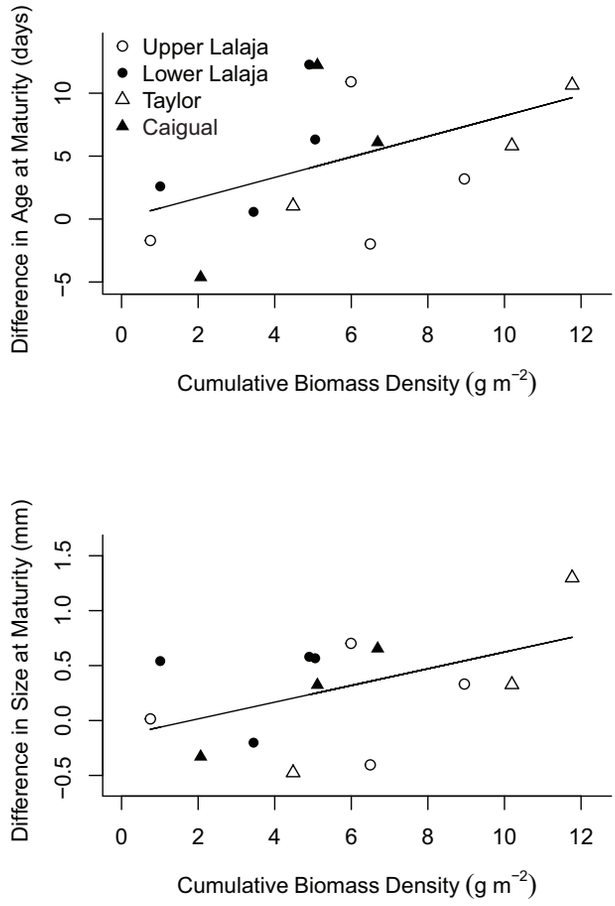

Figure 6: Difference in male age and size at maturity between the introduced and source populations, estimated from the common-garden experiments as in figure 3, as a function of the cumulative biomass density experienced in nature by the grandparents of the fish used to initiate the common garden. Cumulative biomass density was calculated as the sum of the biomass densities corrected for probability of capture in each month prior to the collection of the fish for the common garden. Statistical significance of the correlations was assessed via bootstrap simulations of random pairings of field density and common-garden averages.

Constraints on Response to Strong Immediate Selection. There are three types of constraints that could have produced a lagged response to immediate selection. First, the lag in response to selection might have reflected the waiting time for favorable mutations to emerge. Our observed rates of evolution and the uniformity of response among replicates render this alternative unlikely. Mutational lags could be very long and variable among replicates in populations like ours, which had a relatively small number of founders (78–124) and only two or three generations per year (Reznick et al. 1997), yet evolution occurred very rapidly and concurrently in all four streams. The relatively rapid and uniform response across replicates argues that the evolution was a consequence of standing genetic variation and not new mutations. The fact that prior introductions also produced rapid evolution from different genetic stocks (Reznick and Bryga 1987; Reznick et al. 1990, 1997) bolsters this interpretation.

Second, the lag might instead have reflected the waiting time for a breakdown of the linkage disequilibrium (LDE) associated with a small number of founders and the release of additive genetic variation. We assessed whether LDE could be responsible for the lag in the evolution of male age and size at maturity by estimating the levels of composite LDE between all pairs of our 43 microsatellite loci. These loci are distributed among all chromosomes and thus provide an opportunity to assess composite LDE across the genome. We found no evidence for changing levels of LDE in the period of the experiment, so initial levels of LDE cannot account for the lag in evolution (supplemental material, sec. V, tables S8, S9).

Third, the lag might have appeared because evolution was governed by standing genetic variation in a few genes of large effect, the favorable alleles for which were initially rare. This would be especially true if the advantageous alleles were recessive. In this scenario, the rarity of the advantageous alleles made the initial level of initial additive genetic variance quite low, which in turn caused a limited initial response to selection that was undetectable with our common-garden sample sizes. As the advantageous alleles increased toward intermediate frequencies, the additive genetic variation became sufficiently large for the response to selection to accelerate to the point that we could detect an effect with the sample sizes used in our study.

This is a plausible scenario for male age and size at maturity because genes of large effect have been discovered in other species in the family, sometimes resulting in populations having multimodal size distributions of mature males because each mode represents a different genotype and males grow little after attaining sexual maturity (Smith et al. 2015). The size distribution of mature males in guppy populations generally conform to a normal distribution, but they nevertheless have attributes that suggest the presence of genes of large effect that contribute to male age and size at maturity (Reznick et al. 1997).

Were this scenario responsible for the lag in the response to selection, we ought to have seen a substantial increase in the magnitude of additive genetic variance from the initial generation to the generation represented in the data 2–3 years later, when evolution accelerated. Instead, the additive genetic variance for male size at maturity in LL (the only river for which we currently have a pedigree) was largely unchanged through the first 3 years (T. Potter, personal communication). In the first year of the study, the additive genetic variance was 0.35, yielding an additive genetic coefficient of variation (Houle 1992) of 3.37. In the second and third years, the additive variance was 0.28 and 0.26, respectively, yielding additive genetic coefficients of variation of 3.14 and 3.03. The estimated additive variance was significantly greater than

Table 3: Summary of alternative explanations for the observed lag in the evolution of later age and larger size at maturity in males in the introduced populations of guppies

Mechanism	Test	Result
1. Weak initial selection:		
a. Shape of fitness function: function is normally distributed, so response is initially slow and then accelerates	Initial population growth should be slow and then accelerate as fitness increases	Initial population growth was fast in all four replicates. It slowed and then fluctuated after 2–3 years (fig. 2; supplemental material, sec. I, available online)
b. Small population size: stochastic effects dominate until population size exceeds threshold defined by the magnitude of selection coefficient	Response to selection should be independent of population density once the threshold is exceeded	Response to selection was dependent on population density throughout the study (fig. 6)
2. Constraints on response to strong, immediate selection:		
a. Lag caused by time required for favorable mutations to occur	Given the small number of founders, the duration of the lag should be highly variable among replicates	Lag and subsequent response to selection was the same in each pair of streams (fig. 5; supplemental material, sec. IV)
b. Linkage disequilibrium and the time required for it to break down and release additive genetic variation	If true, there should be changing levels of linkage over time among the microsatellites used to construct the pedigree	There was no change in linkage disequilibrium over time (supplemental material, sec. V, tables S8, S9)
c. Male age/size at maturity controlled by few genes of large effect and initially rare alleles; evolution accelerates as alleles become more common	If true, the additive genetic variation for age and size at maturity should increase over time	Additive genetic variation decreases slightly (from .35 to .26) in the first 3 years
3. Maternal effects: e.g., if older, larger females give birth to males that mature when younger and smaller, causing a delay in the evolution of older and larger size at maturity	Prior studies mimicked the initial conditions of our experiment when testing for maternal effects in response to high resource availability (Bashey 2006)	There was no maternal effect on male age and size at maturity (Bashey 2006)
4. Plasticity: if the plastic response to the initial low densities and high resource availability is to be older and larger at maturity, such plasticity could delay the evolution of older age and larger size at maturity	Is there plasticity in males from high-predation environments to either social environment or food availability?	There is no plasticity in age or size at maturity in high-predation males in response to social environment (Rodd et al. 1997); male size at maturity increases and age at maturity decreases in response to higher food availability (Reznick 1990)
5. Genetic drift	Haphazard differences among replicates in rate and response	All replicates evolved in a similar rate and fashion
6. Sexual selection	Female preference for large males	No such preference is revealed in prior studies (supplemental material, sec. VII)

zero in all years and not statistically distinguishable among years. These values are within the range of values reported for meristic, morphological, and some life-history traits in surveys of evolvability (Houle 1992; Hansen et al. 2011).

Maternal Effects. There could have been a negative maternal effect through which larger, older mothers, who would be favored by an immediate onset of selection, produce sons that mature when younger and smaller (Kirkpatrick and Lande 1989). This maternal effect in the initial generations, when females would carry mostly HP genotypes, would oppose the response otherwise driven by the additive genetic variance for age and size and cause little or no detectable change in the initial generations.

Prior laboratory experiments have shown that when offspring of HP females experience high per capita food levels, as would be the case in the initial generations of our introduction, there are no discernible effects of maternal experience on age and size of maturity of male offspring (Bashey 2006). The lack of maternal influences in laboratory experiments is also reflected in quantitative genetic analyses of variation in male age and size that revealed little latitude for

maternal effects to explain any appreciable proportion of the phenotypic variance in those traits (Reznick et al. 1997) because the heritability of those traits, as estimated from a multigeneration paternal half-sib design, was so high.

Phenotypic Plasticity. The strength of immediate selection could have been weakened if adaptive phenotypic plasticity in response to the high per capita resource levels or the social environment experienced during ontogeny shifted the distribution of phenotypes closer to the optimum (Price et al. 2003). For example, plasticity in the same direction as the selection gradient favoring older ages and larger sizes at maturity could have reduced the difference between the expressed phenotypic mean values and the new optima, thereby weakening phenotypic selection in the initial generations to such an extent that any response was too small to be detected. In this scenario, a response to selection would be detected only when resources or crowding levels were high enough to preclude a plastic response in the same direction as the selection gradient.

Prior experimental evaluations of phenotypic plasticity suggest that plasticity of this type is unlikely to have caused a delay in evolution. First, HP males, which represent the genotypes of the introduced fish, do not exhibit plasticity in maturation in response to their social environment (Rodd et al. 1997). Second, at high per capita resource levels, the plastic response of all males is to mature earlier and larger, not later and larger (Reznick 1990). If there had been an immediate onset of selection for later age and larger size caused by the release from predation, then the plastic response of males to higher per capita resource levels would have increased the selection gradient for later age at maturity but decreased the selection gradient for larger size at maturity. This happens because the plastic response of earlier maturation would have increased the difference between the mean phenotype for age and the new optimum, while larger size would have decreased the difference between the mean phenotype for size and the new optimum. Age and size at maturity in guppies have a high positive genetic correlation (Reznick et al. 1997). This means that whenever there is selection for later age at maturity, the size at maturity will increase as a correlated response and vice versa (Roff 1997, p. 166). Thus, if plasticity in the first generation had enhanced the selection gradient for age at maturity but reduced the selection gradient for size at maturity, there still would have been a substantial response in age and a noticeable correlated response in size. We observed no response in either trait until densities substantially increased.

Finally, there are two additional, broad alternative hypotheses for our results. First, the entirety of the results could have been produced by genetic drift in the breeding values in each population (Hadfield et al. 2010). The relatively small number of founders is consistent with the potential for drift to play a prominent role. Two lines of evidence suggest that this is unlikely. First, assays of genetic variation in the markers used to create our pedigree showed that average heterozygosity decreased minimally through the first 5 years of the experiment and most of the alleles lost were lost in the initial generations. The initial heterozygosity at the 43 microsatellite loci was 0.754 and by year 5 was 0.730. There was little genetic differentiation between the founders and the first cohort: the F_{ST} value between them was 0.019. There were 527 alleles in the founding stock when they were introduced in March 2008. From the first 3 months of 2009 onward, we detected between 400 and 433 alleles in every cohort. These data, along with the rapid ensuing population growth, suggest that, were drift responsible, we would have seen its influence in the earliest months at the lowest densities, whereas we saw mean phenotypes changing in later months at the higher densities. Second, if drift were solely responsible for these results, we would not expect to see the same direction of changes in all four replicates nor the positive correlation between the magnitude of the change and increased population density.

A second alternative is that the evolution of larger body size could have been caused or augmented by sexual selection, especially as density increased. If this hypothesis were true, we would have misdiagnosed the agent of selection. We address this alternative in detail in section VI of the supplemental material. In brief, the considerable body of research on sexual selection in this species does not reveal a consistent preference by females for large males, especially in LP populations.

The Argument for Eco-Evo Feedback

Our results follow Pimentel's (1961) argument for evolution by a genetic feedback mechanism, in that we can show that guppies changed their environment and then adapted to those changes. To demonstrate such an eco-evo feedback loop via density-dependent selection, we need to show (1) that guppies substantially affect their ecosystems and that increased density intensifies those effects, (2) that the effects of those impacts via higher densities actually select for the LP phenotype, and (3) that the outcome of guppy adaptation is a divergence between guppy phenotypes as they adapt to alternative environments. Here we review our prior results demonstrating the first and third points. The new results presented above demonstrate the second point.

Our experiments in artificial streams show that guppies have significant effects on the structure of their ecosystems that are magnified by population density. All guppies deplete their environment of algae and invertebrates, decrease gross primary productivity, and decrease rates of litter decomposition, and they do so to a greater degree at high population densities (Bassar et al. 2010, 2017*a*; Kohler et al. 2012; Travis et al. 2014*b*). These same experiments show that the

nature of the ecosystem impact of guppies from HP and LP environments are quite different from one another (detailed below). Abundant prior results document the adaptive differences between guppies from these alternative environments (Endler 1995). These ecological and evolutionary consequences of guppies indicate that guppy population growth reduces resource availability. These impacts of guppies on ecology create density-dependent selection as the feedback to guppy evolution.

Criteria 1 and 3 for the importance of an eco-evo feedback are thus fulfilled. In addition to the new results presented here, our former publications present suggestive evidence for criterion 2. First, theory predicts that populations that are adapted to higher densities via this eco-evo feedback mechanism ought to display a lessened sensitivity to the depressant effect of increased population density (Engen and Sæther 2016). This is true for guppies: populations from LP, high-density environments are less sensitive to density (Bassar et al. 2013). Second, the effect of guppies on killifish appears to have produced a feedback effect on guppies. Guppies cause a reduction in the population densities of killifish (Walsh et al. 2011; Fraser and Lamphere 2013) and have caused the evolution of killifish life histories (Walsh and Reznick 2011; Walsh et al. 2011). In turn, guppies from LP environments have higher fitness than those from HP environments when killifish are present but not when they are absent (Bassar et al. 2017*b*). The new data presented here further satisfy criteria 2 by providing direct evidence for the role of density in shaping the evolution of the LP phenotype.

All of these results indicate that guppies have shaped their own evolution by shaping their environment. The next question is whether there is evidence of continuing feedback between evolution and ecology. The collective articles on the rotifer-algae interaction document such continuing feedback in the form of repeated population and evolution cycles driven by frequency-dependent selection (Yoshida et al. 2003, 2004). In our case, the system appears to instead be evolving toward a new stable state, yet there is also circumstantial evidence that suggests continuing feedback. The same experiments that quantified the impact of guppies on the ecosystem also show that guppies from LP and HP environments have starkly different effects on their ecosystem. Guppies adapted to HP environments selectively feed on high-quality invertebrate prey and deplete invertebrate abundance. When they graze on algae, they selectively graze on the competitive dominant species and thereby increase algal diversity and primary productivity (Bassar et al. 2017*a*). In contrast, guppies from LP environments feed on invertebrates and algae in proportion to their abundance. Thus, HP and LP guppies cause profoundly different direct and indirect effects on their respective ecosystems (Zandona et al. 2011; Bassar et al. 2013). These results were originally described from short-term experiments performed in natural or artificial streams. They have now been documented when guppies were experimentally introduced above barrier waterfalls (Fraser and Lamphere 2013; Travis et al. 2014*b*; Simon et al. 2017). Results from a different introduction experiment suggest that these differences in foraging are associated with differences in skull and jaw structure (Palkovacs et al. 2011). This combination of results suggests additional steps of feedback between ecology and evolution because the evolution of diet preferences must have followed the impact of guppy population density on resources (eco to evo) but then caused the guppies to evolve in ways that cause them to have very different effects on ecology (evo to eco), presumably enabling them to better exploit the modified ecosystem.

Our results show the evolution of life history in real time in response to changes in population density. They represent a test of a large body of theory, verbal and mathematical, reaching back over 50 years. The initial tests of these theories compared snapshot observations of population variation in life history with snapshot descriptions (often quantitative) of corresponding environmental differences. These tests demonstrated how readily life histories might evolve to match changing environments. What much of that work was unable to demonstrate was why life histories match environments—specifically, which ecological components of those different environments were selecting for different life histories. Many different ecological agents can act selectively on life histories, and some of them, such as predation pressure and population density, are unavoidably correlated with each other. Moreover, the strength of those agents will fluctuate, making snapshot comparisons fraught with the potential to mislead. The net effect is that while we see widespread associations of life history and environment, we cannot always be certain of why those associations occur. Our results suggest that long-term experiments that blend ecology and evolutionary biology may be the only way to achieve anything approaching certainty.

The ecological changes we see in guppy populations with and without predators follow patterns seen in many other ecosystems. In the presence of predators, guppies appear to be a minor component of an otherwise diverse fish community. In the absence of predators, guppies increase in abundance to the point that they play a dominant role in shaping community structure. The changes in community structure wrought by changes in density of a prey species in the absence or presence of its predators is a widespread phenomenon (Travis et al. 2014*a*). Estes et al. (2011) show that anthropogenic disturbances are spreading this phenomenon to diverse ecosystems throughout the world through the systematic elimination of apex predators, which in turn releases prey populations to grow rapidly, resulting in cascading changes to the biological communities and ecosystems. These same changes are occurring in fisheries (Travis and Lotterhos 2013) and in the wake of explosions of invasive species (Simberloff 2010). Our results show that these ecological changes

can also cause rapid evolution of prey with the potential to create not only further cascading ecological changes but rapid cascading evolutionary changes in members of the community. The feedback loops that follow these dynamics can leave us with very different ecosystems and species in very short order.

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APPENDIX

Derivation of Alternative Predictions

Our predictions are based on distinguishing the effects of density-dependent absolute fitness without density-dependent selection from those of density-dependent selection (Travis et al. 2013). To do so, we employ the quantitative genetic approach of Bulmer (1980) on the recursion of mean phenotype as it evolves toward an optimum value. Bulmer's model assumes that fitness is normally distributed around an optimum value, θ, such that the absolute fitness of an individual with phenotypic value z is expressed as

$$\nu(z) = \eta \exp\left(\frac{-(z-\theta)^2}{2\gamma}\right), \tag{A1}$$

where γ is the variance in fitness among phenotypes and η is the maximum fitness. Large values of γ indicate a shallow drop in fitness as z deviates from the optimum (weaker selection), whereas small values indicate a steeper drop (stronger selection).

Density-Dependent Absolute Fitness with Density-Independent Selection

This model describes the situation in which a population encounters selection for a new optimum value, θ, immediately on its introduction into a novel environment. Inserting the fitness function in equation (A1) into a discrete-time model of numerical dynamics without age structure but with density-dependent absolute fitness (population growth) yields

$$n(z, t+1) = n(z,t)\nu(z)e^{-\beta_N \int n(z,t)dz}. \tag{A2}$$

In this model, $N = \int n(z,t)dz$ is total population density. Mean absolute fitness, or the per-time-step rate of growth of the population is density dependent:

$$\bar{\lambda}(N) = \int p(z,t)\nu(z)e^{-\beta_N \int n(z,t)dz}dz = e^{-\beta_N N}\int p(z,t)\nu(z)dz. \tag{A3}$$

The function $p(z,t)$ is the frequency of the phenotypes at time t. Whereas mean absolute fitness, $\bar{\lambda}$, is a function of total population size, mean relative fitness, $\bar{\omega}$, is independent of population size:

$$\bar{\omega} = \int \frac{\nu(z)e^{-\beta_N \int n(z,t)dz}}{\int \nu(z)e^{-\beta_N \int n(z,t)dz}dz}p(z)dz = \int \frac{\nu(z)}{\int \nu(z)dz}p(z)dz. \tag{A4}$$

Both the mean absolute, $\bar{\lambda}$, and the mean relative fitness, $\bar{\omega}$, are functions of the distribution of trait values in the population, $p(z)$.

The selection gradient—the covariance between relative fitness and the trait value—is

$$\begin{aligned}\mathrm{cov}(\omega_i, z_i) &= \int \frac{\nu(z)}{\int \nu(z)dz} zp(z)dz \\ &\quad - \int \frac{\nu(z)}{\int \nu(z)dz}p(z)dz \int zp(z)dz = \overline{\omega z} - \bar{\omega}\bar{z},\end{aligned} \tag{A5}$$

which is also not a function of population size but does depend on both the shape of the fitness function and the distribution of traits in the population at any given time.

Density-Dependent Absolute Fitness with Density-Dependent Selection

By contrast, this model describes a situation in which a population does not encounter a new optimum value on its introduction but sees a new optimum value emerge as its density increases. In the simplest case, θ is a linear function of total density:

$$\theta(N) = \theta_N N, \tag{A6}$$

which also makes the fitness function a function of density:

$$\nu(z, N) = \eta \exp\left(\frac{-(z-\theta(N))^2}{2\gamma}\right). \tag{A7}$$

To keep $\theta(N)$ within a reasonable range, we set $\theta_N N$ to have a maximum value of 1, which, in our parameterization of the model, we take to represent the LP phenotype.

Inserting the fitness function (A7) into the population dynamic model yields

$$n(z, t + 1) = n(z, t)\nu(z, N)e^{-\beta_N \int n(z,t)dz}. \quad \text{(A8)}$$

In this model, absolute fitness, $\bar{\lambda}$, is density dependent:

$$\bar{\lambda}(N) = e^{-\beta_N N} \int p(z, t)\nu(z, N)dz, \quad \text{(A9)}$$

but in contrast to the density-independent selection model, relative fitness, $\bar{\omega}$, is also density dependent:

$$\bar{\omega}(N) = \int \frac{\nu(z, N)}{\int \nu(z, N)dz} p(z)dz. \quad \text{(A10)}$$

Likewise, the selection gradient now also depends on population density,

$$\begin{aligned} \mathrm{cov}(\omega_i, z_i) &= \int \frac{\nu(z, N)}{\int \nu(z, N)dz} zp(z)dz \\ &\quad - \int \frac{\nu(z, N)}{\int \nu(z, N)dz} p(z)dz \int zp(z)dz \quad \text{(A11)} \\ &= \overline{\omega(N)z} - \overline{\omega(N)}\bar{z}. \end{aligned}$$

Model Predictions

To illustrate the dynamics of the model, we chose reasonable values for the parameters and iterated the population through 48 time steps (months). The population begins with a mean phenotype value equal to 0. In the density-independent case, the optimum in the new environment is $\theta = 1$. In the density-dependent case, the optimum in the new environment is 0 initially but increases steadily to 1 as population density increases. The parameters were set as follows: $\eta = 2$, $\gamma = 2.2$, $\theta = 1$, $\theta_N = 0.0002$, $\beta_N = 0.0001$. Assume for simplicity that the initial distribution of phenotypes in the population is also Gaussian with a mean of 0 and a variance δ:

$$n(z, t = 0) = \exp\left(\frac{-(z - 0)^2}{2\delta}\right). \quad \text{(A12)}$$

The area under this curve is the initial population size. For the illustration, we set $\delta = 1$ and, for the density-dependent scenario, used two starting densities.

The temporal trajectories of the selection gradient, the immediate response to selection, and the cumulative trait change are qualitatively different between the models of density-independent and density-dependent selection (fig. 1*A*–1*C*), even though the trajectories of population density are not (fig. 1*D*). At a starting density near 0 in the case of density-independent selection, the population's mean phenotype is on the left-hand side of the fitness function and selection is immediately strong (eq. [A5]; fig. 1*B*). The response to selection is also immediately large (fig. 1*C*). As the mean phenotype moves toward the optimum, the selection gradient decreases, the response to selection decreases, and the increase in the mean phenotype slows continuously with time.

By contrast, when selection is density dependent, the population's mean phenotype is near or at the optimum for very low density, the selection gradient is small (eq. [A11]; fig. 1*B*), and the immediate response to selection is negligible (fig. 1*C*). When the population begins to grow, the optimum phenotype shifts to the right, beginning its movement toward $\theta = 1$. The population's mean phenotype is now below the optimum, and selection begins. At very low densities, if the mean phenotype is above 0, there may be initial selection toward the initial optimum of 0 (fig. 1). As population growth accelerates, the selection gradient rapidly becomes larger (fig. 1*B*), the response to selection increases (fig. 1*C*), and the cumulative change in mean phenotype accelerates (fig. 1*A*).

A critical element in this model is the magnitude of the variances in the fitness function and the initial phenotype distribution. In section I of the supplemental material, we show how the predictions of the density-independent model depend on these variances and justify our choices of parameter values as reflections of what we know about guppies.

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Guppies teach us how and why evolution happens. Artwork credit: Tess Reznick.