LIFE-HISTORY EVOLUTION IN GUPPIES (*POECILIA RETICULATA*): 1. PHENOTYPIC AND GENETIC CHANGES IN AN INTRODUCTION EXPERIMENT

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Abstract.—Previous investigations (Reznick and Endler, 1982; Reznick, 1982*a*, 1982*b*) demonstrated that genetic differences in guppy life histories were associated with differences in predation. Guppies from localities with the pike cichlid *Crenicichla alta* and associated predators matured earlier, had greater reproductive efforts, and produced more and smaller offspring than did guppies from localities with only *Rivulus harti* as a potential predator. *Crenicichla* preys primarily on large, sexually mature size-classes of guppies, while *Rivulus* preys primarily on small, immature size-classes. These patterns of predation are hypothesized to alter mean age-specific survival. Theoretical treatments of such differences in survival predict the observed trends in age at maturity and reproductive effort.

We are using introduction experiments to evaluate the role of predators in selecting for these life-history patterns. The experiment whose results are presented here was conducted in a tributary to the El Cedro River (Trinidad), where a waterfall was the upstream limit to the distribution of all fish except *Rivulus*. Guppies collected from the *Crenicichla* locality immediately below the waterfall (the downstream control) were introduced over the waterfall in 1981. This introduction released the guppies from *Crenicichla* predation, exposed them instead to *Rivulus* predation only, and also introduced them to a different environment, since the introduction site has greater canopy cover than the site of origin. Changes in guppy life-history patterns can be attributed to predation and/or the environment.

Evidence from fish collected and preserved in the field demonstrated that, by mid-1983, guppies from the introduction site above the waterfall matured at larger sizes and produced fewer, larger offspring. There were no consistent differences in reproductive allotment (weight of offspring/total weight). With the exception of reproductive allotment, these patterns are identical to previous comparisons between *Rivulus* and *Crenicichla* localities. A laboratory genetics experiment demonstrated that males from the introduction site matured at a later age and at a larger size than did males from the control site downstream, as predicted from the "age-specific predation" hypothesis. No differences between localities were observed for female age and size at maturity or for reproductive effort. The trends for fecundity and offspring size were the reverse of those observed in the field. Because only the males changed in the predicted fashion, it is not possible either to reject or to accept the hypothesis of age-specific predation at this time. We discuss the possible causes for these patterns and the high degree of plasticity in the life history, as evidenced by the differences in fecundity and offspring size between the field and laboratory results.

Empirical testing of evolutionary theories is a challenge because the time required to evolve generally exceeds the practical limits of experiments. One solution for testing specific theories involves artificial selection experiments with laboratory populations. Such studies evaluate the plausibility of a given mode of evolution but not its importance in nature (Endler, 1986). Furthermore, most natural populations are confronted by a diversity of potentially interacting influences; understanding why organisms are the way they are requires an understanding of these interactions. A second solution, but one which is rarely available, involves finding the appropriate circumstances in natural populations where selection can be manip-

ulated and where the target organisms can respond within a reasonable period of time.

Life-history evolution has attracted much interest in the past two decades. Progress has been made in the development of theory and the demonstation of life-history variation in natural populations (reviewed by Stearns [1976, 1977]). Only recently have investigators experimentally tested the predictions of life-history theories (Solbrig and Simpson, 1977; Luckinbill, 1978, 1979, 1984; Taylor and Condra, 1980; Barclay and Gregory, 1981, 1982; Doyle and Hunte, 1981; Mueller and Ayala, 1981; Rose and Charlesworth, 1981; Luckinbill et al., 1984; Rose, 1984). These experiments have mostly tested the predictions of two theories:

1) r- and K-selection or 2) selection due to age-specific mortality. Testing r- and K-selection has generally involved manipulating population density, while testing age-specific mortality has generally involved reducing adult lifespan. The results are mixed and, if taken at face value, do not strongly support the predictions of either theory. However, some of these studies have confounded the two modes of selection (Reznick, 1985). For example, in some cases density was manipulated by selectively removing adults, so density and age-specific survival were changed simultaneously. Studies that manipulate only survival (e.g., Rose and Charlesworth, 1981) support predictions based on theories of age-specific mortality. Furthermore, Stearns (1977), Kozlowski (1980), and Boyce (1984) have argued that many of the predictions attributed to r- and K-selection are not justified by the theory, so it cannot be said whether the results of a given experiment do or do not support the theory. The positive aspect of these experiments is that they demonstrate that most organisms have the necessary genetic variation to respond to demographic manipulations.

We have the similar goal of evaluating models of life-history evolution, but our project differs from previous studies because it considers a form of natural selection (predation) in a field experiment. A consequence of our design is that we are not testing a specific theory of life-history evolution, since predation can alter life histories in several ways. A virtue of the design is that it will ultimately allow us to evaluate the importance of different modes of evolution, plus their interactions, in natural populations. We feel that the theories of r- and K-selection and of age-specific mortality are not exclusive alternatives; different modes of selection may act simultaneously.

Natural populations of guppies (*Poecilia reticulata*) from the Northern Range of Trinidad have life-history differences associated with differences in predation (Reznick and Endler, 1982; Reznick, 1982a, 1982b). In one series of localities guppies co-occur with the pike cichlid *Crenicichla alta* plus other predators, while in a second series of localities the killifish *Rivulus harti*

is the only important predator. *Crenicichla* and some of the associated species of predators prey predominantly on large, sexually mature size-classes of guppies (Seghers, 1973, 1974; Liley and Seghers, 1975). *Rivulus* preys predominantly on small, immature size-classes of guppies (Seghers, 1973; Liley and Seghers, 1975). The size specificity of these predators suggests that they could select for life-history changes in guppies by altering age-specific survival: by preying on large guppies, *Crenicichla* should reduce adult survival; by preying on small guppies, *Rivulus* should reduce juvenile survival.

Theoretical treatments of age-specific mortality predict that reduced adult survival will select for individuals that mature earlier and have greater reproductive efforts (Gadgil and Bossert, 1970; Law, 1979; Michod, 1979; Charlesworth, 1980). The opposite response is predicted for reduced juvenile survivorship. The genetic differences in guppy life histories between these two types of localities (Reznick, 1982a, 1982b, unpubl.) correspond to theoretical predictions. Guppies that co-occur with *Crenicichla alta* and other predators mature at an earlier age and have higher reproductive efforts than guppies that co-occur with just *Rivulus harti*. Furthermore, guppies from *Crenicichla* localities tend to produce more and smaller offspring than do their counterparts from *Rivulus* localities.

Factors other than predators may also have selected for these patterns. Differences in predation are associated with differences in habitat and guppy density. *Rivulus* localities are generally smaller streams with more canopy cover and higher densities of guppies (Seghers, 1973; Liley and Seghers, 1975; Endler, 1978; Reznick and Endler, 1982). The heavy canopy cover reduces light level and probably primary productivity; such a reduction in productivity has been demonstrated for temperate-zone streams (Hawkins et al., 1983). Since guppies feed in part on algae and bacteria scraped from environmental surfaces (Dussault and Kramer, 1981), such a reduction in productivity would directly affect food supply. Reduced primary productivity and higher population densities may mean less food for guppies. The resulting density effects could be an in-

dependent source of selection (MacArthur and Wilson, 1967; Boyce, 1984). One general response to such selection is reduced allocation of resources to reproduction (reviewed by Boyce [1984]); differences in guppy life histories are also consistent with this prediction. Finally, there may be an interaction between predation and the environment. For example, the increased density of guppies in *Rivulus* localities may be caused in part by the accumulation of individuals who have outgrown their predators; density effects may thus be mediated in part by predators.

Disentangling these different forms of causality requires field experiments. We report here the results of such an experiment. Briefly, we found a locality where a waterfall was the upstream border to the distribution of all fish except *Rivulus harti*; below the waterfall we found guppies, *Crenicichla,* and a variety of other species. *Rivulus harti* was also present below the waterfall, but at far lower densities. Guppies from below the barrier waterfall were introduced over the waterfall. Introducing guppies above the barrier waterfall released them from predation by *Crenicichla* and associated predators. The expected change in age-specific survival should result in selection for guppies that are older at maturity and have lower reproductive efforts relative to guppies from below the barrier waterfall (Gadgil and Bossert, 1970; Law, 1979; Michod, 1979; Charlesworth, 1980). We recognize that other sources of selection may be associated with this introduction and discuss these below. In addition, our previous comparisons of guppies from *Rivulus* versus *Crenicichla* localities (Reznick and Endler, 1982; Reznick, 1982a, 1982b) predict that guppies from the introduction site should produce fewer and larger offspring than do those from the downstream control.

We first assessed the response to the introduction by characterizing the life-history phenotypes of wild-caught guppies from the introduction site and the downstream control site. Our conclusions about evolutionary changes in the life history are based on a laboratory genetics experiment conducted at the University of California, Riverside. We studied the second laboratory-reared generation, in order to control for any en-

vironmental influences on the life history. We included high and low levels of food availability because we suspect that there are natural differences in resource availability among localities (Reznick and Endler, 1982). If resource availability has been important in selecting for life-history changes, then the expression of genetic differences in life histories may depend on the level of resources in the laboratory study.

MATERIALS AND METHODS

The general biology of guppies is summarized elsewhere (Haskins et al., 1961; Seghers, 1973; Liley and Seghers, 1975; Endler, 1978; Reznick and Endler, 1982; Endler, 1983) so we only give a few details about the guppy life cycle. Males and females usually mature when they are 15–20 mm, standard length. There is a pronounced sexual dimorphism in size and color. Male growth is determinate, with little growth after maturation, while females grow continuously and, hence, attain larger sizes. Males have bright, highly polymorphic color patterns; females have uniform, inconspicuous coloration. The generation time, estimated as the interval between when a female is born and when she gives birth to her first litter, varies from 10 to 20 weeks in the laboratory (25°C), depending on food availability. Guppies are viviparous and produce litters at approximately 25-day intervals in the laboratory.

Introduction Experiment. —Our design exploits the natural distribution of guppies and their predators and is similar to earlier introduction experiments (Endler, 1980, 1983; Reznick and Endler, 1982). Some Northern Range streams have waterfalls, which serve as an upstream barrier to the distribution of guppies and/or their predators (first described by Haskins et al. [1961]). The current experiment is on a tributary of the El Cedro River. The barrier is a series of cataracts, capped at the upstream end by a five-meter free fall through a narrow spout. Our conclusion that only *Rivulus* occurs above the barrier came from three extensive surveys, two extending approximately 1 km upstream, over a six-week period in February and March 1981. Most species of fish in these streams, especially guppies, can be spotted within a few minutes of observa-

tion, so failure to see them in our surveys can be taken as evidence of their absence. Guppies are particularly conspicuous because they are diurnal, swim in the open, and make no attempt to hide when disturbed. The absence of other species was confirmed with eight subsequent surveys of the locality.

The portion of the river below the barrier waterfall was included in an earlier survey of guppy life histories (Reznick and Endler, 1982) as the El Cedro 1 locality (=the downstream control site in the present study). The profile of the stream is atypical of our *Crenicichla* localities because it is smaller and of lower order, has a lower flow rate, and has a higher population density of guppies (see Reznick and Endler, 1982 table 1). It is similar to most *Rivulus* localities in these regards. The life history patterns of the guppies from this sample were similar to our other *Crenicichla* localities, except that mean offspring size was relatively large (Reznick and Endler, 1982 table 2). The El Cedro *Rivulus* locality reported in the earlier paper (El Cedro 3) represents a different tributary from the one chosen for this experiment.

The complete experiment involves two introductions; we here report on the first introduction. In this first phase, guppies from the *Crenicichla* locality below the barrier (the downstream control) were introduced over the waterfalls. This introduction changed the predator from *Crenicichla* to *Rivulus,* but also changed the habitat, since the introduction site has a heavier canopy cover than the downstream control site. Other aspects of the introduction site, such as carbonate hardness, pH, and water temperature are very similar to those of the downstream control site (Table 1), and the approximate stream dimensions were, likewise, very similar. The introduction site is also similar to the downstream control site in having large, deep pools, which *Crenicichla* seem to prefer as home ranges and breeding sites. If the guppies adapt to the new environment, then the next phase of the experiment will be to introduce *Crenicichla* above the barrier waterfall.

The guppy introduction was made on 16 March 1981. Approximately 100 fish, including many large, gravid females, were

TABLE 1. Water conditions at the introduction and downstream control sites. Values are means of readings taken in June 1983, Feburary 1984, and April 1985. Temperature range is also shown (in parentheses).

	Site	
Variable	Downstream control	Introduction
Temperature (°C)	24.4 (23–26)	24.0 (23–26)
pH	7.6	7.5
Carbonate hardness (ppm)	98.5	89.6

collected from the *Crenicichla* locality below the barrier waterfall and introduced above the fall. The genetic diversity of the introduced population was probably greater than indicated by the number of introduced individuals, because females store sperm and are generally multiply inseminated, as in natural populations of other species of Poeciliids (Borowsky and Kallman, 1976; Borowsky and Khouri, 1976). We introduced approximately equal numbers of fish to four pools over approximately 0.5 km of the stream. We visited the introduction site in August 1981 and July 1982. On both occasions, we found a large population, which had extended its range downstream to the barrier waterfall and upstream to a second series of cataracts. Guppies did not disperse over the new upstream border in later visits, through April 1985.

Processing of Preserved Materials. —We collected and preserved guppies from the introduction site and the downstream control, beginning 27 months after the introduction, to estimate life-history variables; these collections were made in June 1983, February 1984, and April 1985. Field-collected females were sampled to give an even representation of all millimeter size-classes. The variables measured included 1) standard length to the nearest 0.05 mm, 2) somatic dry weight, 3) fecundity (the number of developing offspring), 4) offspring stage of development, 5) offspring size (the average dry weight of offspring), 6) "Reproductive allotment" (offspring weight/[offspring weight plus somatic dry weight]), 7) the minimum size class of reproducing females (the smallest size-class in which the majority of females had yolking ova), and 8) the average size of mature males. Ma-

turity was judged on the basis of anal-fin morphology (Turner, 1941).

Laboratory Methods. — In April 1985, 23 gravid females were collected from the introduction site and the downstream control site. Based on laboratory determinations of generation time, our best estimate is that these fish were collected after 10–20 generations of selection. To minimize the effect of microenvironmental trends within the laboratory, we assigned fish to tanks, by locality, in a stratified randomized fashion in this and in subsequent generations. Each fish was isolated in an 8-liter tank, and her offspring were collected as the first laboratory generation. The progeny of each female were considered to represent distinct lineages. All young were raised at a density of 10 per 19-liter tank until they were 25 days old. They were then still immature but could be sexed by noting the presence of melanophores at the base of the abdomen or changes in the anal fin (Turner, 1941). Sexes were separated and raised until the first-laboratory-generation females were large enough to bear at least ten young (approximately 24 mm long and 300 mg).

First-generation females were mated with first-generation males from a different lineage but the same locale to produce the second laboratory generation. Nearly all lineages were represented in the crosses by one male and one female, and all crosses represented a unique combination of lineages. Because some parental females produced only male or female offspring, only 22 of the original 23 lineages were included in the first laboratory generation from the introduction site. We used a wild-caught male to mate to one first-generation introduction-site female to compensate for the absence of first-generation males in one lineage.

The offspring of these crosses, the second laboratory generation, were reared in the same fashion as the first laboratory generation until they were 26–31 days old. Every individual in each litter was then weighed and measured. We selected two males and two females from the middle of the size distribution in each sib-group to continue in the quantified feeding phase of the study. The remaining young were preserved. Within sib-groups, each male was randomly paired with one of the two females in a glass-partitioned 8-liter tank, with the female in the front half. One pair in each sib-group was assigned to a "high" food level, and the other pair was assigned to a "low" food level. Controlled feeding followed the methods of Reznick (1983), except that all food levels were systematically lower in this study. The two tanks with the two sib-pairs were placed beside each other on the shelf. The placement of these pairs of tanks on the shelves was randomized by locality.

Males were observed weekly for sexual maturity; those close to maturity were checked every 3–4 days. Decisions about maturity were based on hood and hook development in the gonopodium (after Turner [1941]). To minimize bias in deciding when males were mature, one observer placed all males that were mature or close to mature in coded containers; a second observer, without a knowledge of the origin of the male, decided which fish had completed development. We preserved the mature males.

Second-generation females were mated once a week with a randomly chosen wild-caught male, beginning while they were immature, until first parturition. Females are highly receptive to mating within 24 hours of bearing young (Liley, 1968); they were crossed again at this time. Males were placed in the tank after the last feeding of the day and removed before the first feeding the next morning to prevent interference with controlled food availability. The partitions were removed from "high" and "low" food tanks six and eight weeks after the initiation of controlled food availability, respectively. Most of the male siblings had matured and been preserved by this time. The 19 males that had not yet matured (out of 86 total) were transferred to equivalent 8-liter aquaria. After removing the partition, we placed a net approximately 5 cm from the rear of each female's tank. The mesh size allowed offspring to pass through while keeping a mature female out, thus reducing the opportunity for cannibalism. Offspring, the third laboratory generation, were collected within 12 hours of birth and preserved. Females were preserved immediately after bearing their second litter, and later dissected to find unborn or cannibalized young. Only one of 85 females had eaten any of her offspring (a single newborn fish).

The variables measured in this study included 1) age, weight, and standard length of second-laboratory-generation males at maturity, 2) age, weight, and length of second-generation females after first and second parturition, 3) interbrood interval (number of days between litters), 4) number of offspring in the first and second litters (fecundity), 5) mean dry offspring weight in each third-generation litter, 6) "reproductive allotment" for the second litter, and 7) "reproductive effort" for the entire term of the experiment (as described in Reznick [1983].

Data Analysis.—The field results were analyzed as a one-way analysis of variance, with a separate analysis for each collection date, using the SAS GLM procedure (SAS Inst., 1985). Female somatic dry weight and the stage of development of offspring were used as covariates in the analysis of offspring weight and reproductive allotment. Female somatic dry weight was a covariate in the analysis of fecundity. Slope homogeneity was confirmed, using the SAS GLM procedure, before a covariate was included in the model.

The laboratory results were analyzed as a two-way analysis of variance with locality and food availability as fixed main effects. The weight of the second-generation offspring at the beginning of controlled feeding, the date that they entered the experiment, the average water temperature of each tank, and the number of days when a fish did not eat its full food ration were evaluated as potential covariates for all dependent variables. In addition, we considered the postpartum wet weight of a female as a potential covariate for the number and size of offspring. We screened the potential significance of the covariates by first performing a nonstepwise multiple linear regression within each locality and food-availability treatment and for each dependent variable. Covariates with the same sign for the regression coefficients in all four treatment groups were then evaluated for slope homogeneity and significance as part of the two-way analysis of variance before they were included in the final analysis.

The assumptions of all analyses were evaluated with analyses of the residuals. The normality of residuals was tested with the
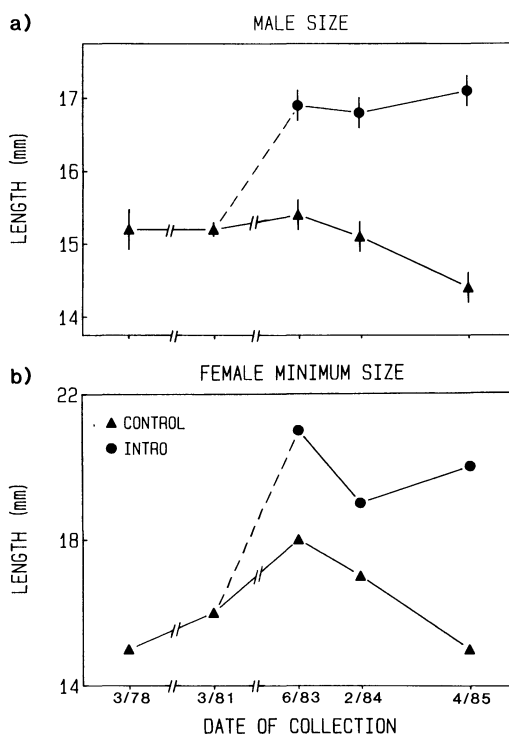

FIG. 1. Field phenotypes in the El Cedro River. The introduction-site guppies (circles), first sampled in June 1983, were derived from the downstream-control site (triangles) in March 1981. A dashed line joins the March 1981 downstream-control data with the June 1983 introduction-site data to indicate the source and timing of the introduction. a) Mean standard length ($\pm$SE) of sexually mature males. Values for June 1983, February 1984, and April 1985 are least-square means from the analyses reported in Table 2. b) Minimum size class (mm) of reproducing females (see Materials and Methods for definition).

SAS Proc Univariate. Homogeneity of variance was evaluated first with a plot of residuals versus predicted values, then with an F_{max} test (Sokal and Rohlf, 1981). The data for some dependent variables were log-transformed to normalize the distribution of the residuals. The assumptions of normality and homogeneity of variance were satisfied for all of the reported results.

RESULTS

Field Samples.—Our three pairs of field samples (June 1983, February 1984, and April 1985) recorded the life-history phenotypes for the two localities and provided the first clues of evolved changes in life histories. Figures 1 and 2 include data for 1978

TABLE 2. Summary of analyses of variance of life-history variables in field-collected fish. Definitions of dependent variables: "fecundity" = number of developing embryos in females; "offspring weight" = mean dry weight of developing embryos in females; "reproductive allotment" = dry weight of developing offspring/total dry weight; "male size" = standard length (mm) of sexually mature males. Definitions of covariates: "female weight" = somatic dry weight (mg) of sexually mature females; "developmental stage" = the stage of development of the embryos. Values recorded for "locality" and covariates are F ratios. Also reported are the residual sums of squares and residual degrees of freedom.

Variable	Source	June 1983	February 1984	April 1985
Fecundity[a]	Female weight (covariate)	28.9**	46.7**	140.4**
	Locale	4.0†	46.0**	15.4**
	Residual SS (d.f.)	60.65 (27)	207.99 (36)	167.02 (42)
Offspring weight[b]	Developmental stage (covariate)	9.4*	—	—
	Female weight (covariate)	—	7.97**	13.5**
	Locale	28.2**	139.53**	48.9**
	Residual SS (d.f.)	2.58 (27)	0.1337 (36)	2.43 (42)
Reproductive allotment	Developmental stage (covariate)	4.9*	5.4*	8.6**
	Female weight (covariate)	—	7.2*	12.8**
	Locale	0.8	16.3**	0.4
	Residual SS (d.f.)	0.0607 (27)	0.0809 (35)	0.0724 (41)
Male size	Locale	23.2**	31.0**	96.7**
	Residual SS (d.f.)	36.36 (38)	37.53 (38)	27.60 (38)

[a] Slopes of female weight-fecundity regressions were not homogenous for the June 1983 and April 1985 collections.
[b] Data were log transformed for the February 1984 collection.
† $0.05 < P < 0.10$.
* $P < 0.05$.
** $P < 0.01$.

and 1981 collections from the downstream control site, illustrating the stability of all life-history variables before the introduction.

Mature males from the introduction site were significantly larger than those from the downstream control site on all three collection dates (Table 2, Fig. 1). The minimum size class of reproducing females displayed the same trends (Fig. 1). Since female minimum size is represented by only a single number in each sample, this variable was not amenable to statistical analysis. In our previous work, these size statistics served as accurate indices of the age at maturity, implying that fish from the introduction site mature later than those from the downstream control site.

Two indices of reproductive effort are reproductive allotment and interbrood interval. Since guppies provide no postpartum care for their young, these two variables include much of the natural variation in reproductive effort. We found no consistent differences between localities for reproductive allotment (Table 2, Fig. 2); the two sites did not differ significantly in the June 1983 collection, and the downstream control site had higher values in February 1984, but lower values in April 1985. Data on inter-

brood intervals were only available from the parental females in the laboratory genetics study. The two populations did not differ for this variable ($F_{[1, 38]} = 0.0854$, ns; introduction site: $\bar{x} = 25.9$ days; downstream control site: $\bar{x} = 26.0$ days). The results for the two variables imply that females from these localities do not differ in reproductive effort.

Finally, the introduction-site guppies produced fewer and larger offspring than did their downstream control counterparts (Table 2, Fig. 2). The magnitudes of the differences and the means for each locality varied across time, but the results were consistent and highly significant in all three sampling periods.

In summary, the differences in life-history phenotypes in wild-caught fish parallel those for our previous comparisons between *Rivulus* and *Crenicichla* localities for all variables except indices of reproductive effort. Introduction-site guppies were larger at maturity and produced fewer, larger offspring per brood. Such phenotypic differences are not necessarily genetic. The purpose of the laboratory study was estimation of the genetic basis for these observations.

Laboratory Genetics Experiment. — The second-generation laboratory-reared males

a)

REPRODUCTIVE ALLOTMENT

a)

MALE AGE AT MATURITY

b)

FECUNDITY

b)

MALE WEIGHT AT MATURITY

c)

OFFSPRING SIZE

DATE OF COLLECTION

FIG. 3. Male age (±SE) and live weight (±SE) at maturity in second-generation, laboratory-reared guppies. Values are least-square means from a two-way analysis of untransformed data.
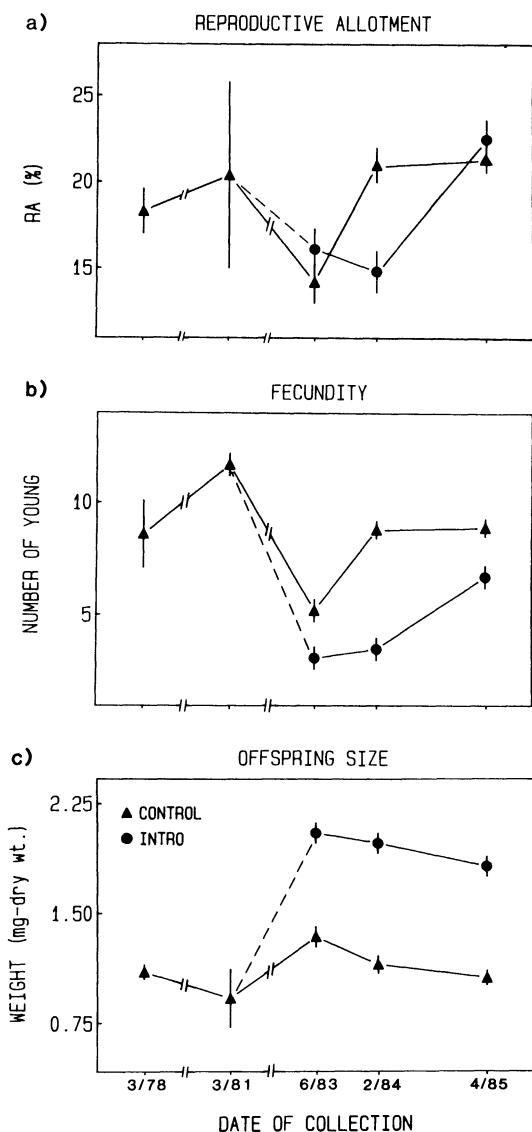
FIG. 2. Mean field phenotypes (±SE) in the El Cedro River: a) reproductive allotment in females with developing embryos (see Materials and Methods for definition); b) number of developing offspring; c) offspring weight. The symbols and the dashed line are explained in the legend to Figure 1. Values for June 1983, February 1984, and April 1985 for all three dependent variables are least-square means from the analyses reported in Table 2.

magnitude of the difference between the two localities was larger at low levels of food availability. In general, low food males were older and smaller at maturity than high-food males.

Second-generation females from the two localities did not differ in the age or size at first parturition (Table 3, Fig. 4). There was a significant effect of food availability which paralleled the results for males; low food females were older and smaller at first parturition than their high food counterparts. There was a significant interaction between locality and food availability for age and a marginally significant interaction for weight. Such interactions indicate some differences between the two groups, but we cannot offer an interpretation for the pattern. The differences between localities at high food were similar to the results for males; down-stream-control females produced their first litter when they were smaller and younger than introduction-site females (Fig. 4). However, the relative rankings of the two localities were reversed in the low-food treatment; females from the downstream

from the introduction site were significantly older and larger at maturity than their counterparts from the downstream-control site (Table 3, Fig. 3). We found a significant food-by-locality interaction, because the
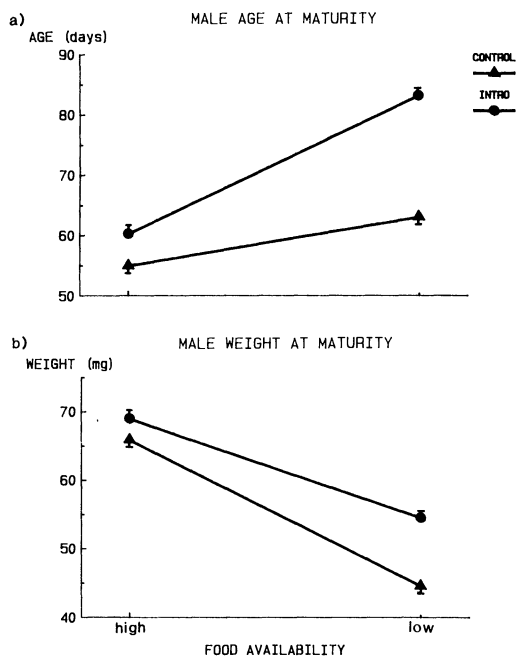
TABLE 3. Analyses of variance of life-history variables in fish reared in a common laboratory environment for two generations. Table entries are F values. Definitions of dependent varibles: "male age" and "male weight" = age (days) and wet weight (mg) at maturation; "female age" and "female weight" = female age (days) and wet weight (mg, postpartum) at first parturition; "interval" = time interval (days) between the birth of the first and second litters; "RA2" = reproductive allotment (%) for the second litter; "RE" = reproductive effort for the full course of the experiment; "N1" and "N2" = the number of offspring in the first and second broods, respectively; "offspring wt1" and "offspring wt2" = mean dry weight (mg) of individual offspring in the first and second litters. Definitions of covariates: "initial weight" = the wet weight (mg) of the fish at the beginning of the experiments; "date in" = the calendar date when a block was initiated; "no eat" = the number of days during the experiment when a fish did not eat all available food; "wt1" and "wt2" = the postpartum wet weights (mg) of a female after the first and second litters, respectively. See text for further details.

Source	Male age[a]	Male weight[a]	Female age[a]	Female weight[a]	Interval[b,c]
Covariates:					
Initial weight	21.43**	8.59**	—	—	—
Date in	—	—	—	6.81**	—
No eat	—	—	5.76*	—	—
Wt1	—	—	—	—	—
Wt2	—	—	—	—	—
Main effects:					
Locality	25.72**	12.39**	0.98	0.00	0.11
Food	43.94**	83.14**	206.98**	63.99**	1.36
Locality × food	5.73*	5.06*	5.16*	3.02†	1.62
Residual (d.f.)	0.383 (81)	0.385 (81)	0.199 (81)	3.478 (81)	432.28 (80)
R^2	0.57	0.56	0.75	0.48	0.04

[a] Analysis performed on log-transformed data.
[b] One outlier deleted. This individual had a 46-day interval between litters, which is approximately two times the expected value and was interpreted as a skipped litter.
[c] One individual jumped out of its tank and died between the first and second litter, accounting for a lost residual degree of freedom.
† $0.1 < P < 0.05$.
* $0.05 < P < 0.01$.
** $P < 0.01$.

control site tended to be larger and older at first parturition than their introduction-site counterparts. The net effect of the interaction was no difference between locality means. There were no significant effects of locality, food availability, or their interaction on reproductive allotment, interbrood interval, or reproductive effort (Table 3, Fig. 5).

Fecundity and offspring weight were analyzed separately for the first and second litters (Table 3, Fig. 6). The results were qualitatively similar, so only the second litter is included in Figure 6. The trend in both litters was for the introduction-site females to produce more and smaller offspring than did females of the same size from the downstream-control site; note that these trends are the opposite of those observed in the wild-caught fish. Locality effects were not significant for the number or size of offspring in the first litter. Locality had a marginally significant effect on fecundity in the second litter ($0.05 < P < 0.10$) and a sig-

nificant effect on offspring weight in the second litter ($P < 0.05$). Lower food availability caused significantly larger offspring in both the first and second litters, plus significantly fewer offspring in the second litter.

To summarize, the results for male age and size at maturity and for reproductive effort in second-generation fish are consistent with our field observations: introduction-site males were older and larger at maturity, while reproductive effort did not differ in the two localities. Fish from different localities also did not differ in female age or size at maturity, which is contrary to the field observations. Finally, there was a reversal in the relative fecundity and offspring size between the field phenotypes and the genetic differences expressed in the lab. The introduction-site guppies tended to produce more and smaller offspring in the second laboratory generation than did guppies from the downstream-control site. Reduced food availability resulted in later maturity at a

TABLE 3. Extended.

Dependent variables					
RA2^c	RE	N1	N2	Offspring wt1^c	Offspring wt2^c
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	62.17**	—	15.09**	—
—	—	—	49.37**	—	4.77*
0.88	0.68	2.12	2.92†	2.41	6.91*
1.13	0.40	0.84	5.96*	33.98**	19.08**
1.37	0.71	0.14	0.73	0.39	0.34
0.135 (81)	66.85 (81)	136.55 (81)	246.02 (80)	1.583 (81)	1.120 (80)
0.04	0.02	0.54	0.61	0.31	0.25

smaller size, lower size-specific fecundity, and increased offspring size.

DISCUSSION

The Response to Selection and Potential Causes.—Our introduction experiment tests the hypothesis that a combination of predators and the environment has selected for the observed interpopulation differences in guppy life histories. A more specific hypothesis is that these patterns are caused by age-specific predation. Our introduction moved guppies from an area with selective predation on adults to an area with selective predation on juveniles. We predicted that the introduction will favor individuals that mature later and have lower reproductive efforts. The results for males fulfill the predictions, while the results for females do not (Fig. 7). It is thus not possible to accept or reject the hypothesis at this time.

It is possible that males respond more rapidly to selection than do females; the strength of selection may be greater for males, males may have more genetic variation for age at maturity, or the sexes may differ in the covariance of age and size at maturity with other aspects of the life history. Genetic covariances could restrict the rate of response to selection. These factors, plus others that would govern the rate of response to selection (field generation time or the overall coefficient of selection) are unknown, so it is impossible to decide in advance when to terminate the experiment. This is in contrast to introduction experiments by Hairston (1980, 1986), in which a termination date was specified in advance, based on prior knowledge of the generation time. If the sexes respond to selection at different rates, then the prediction is for a change in females in future assays of these populations.

An unexpected response to selection was the apparent reduction in offspring size and increase in fecundity in the introduction site; all previous results imply that selection should favor the opposite response. If density and resource availability are important sources of selection in this system, then it is possible that this trend is the result of the transient influence of an expanding population. The short-term effects of density would thus be the opposite of what one would predict for general comparisons between *Rivulus* and *Crenicichla* localities. If
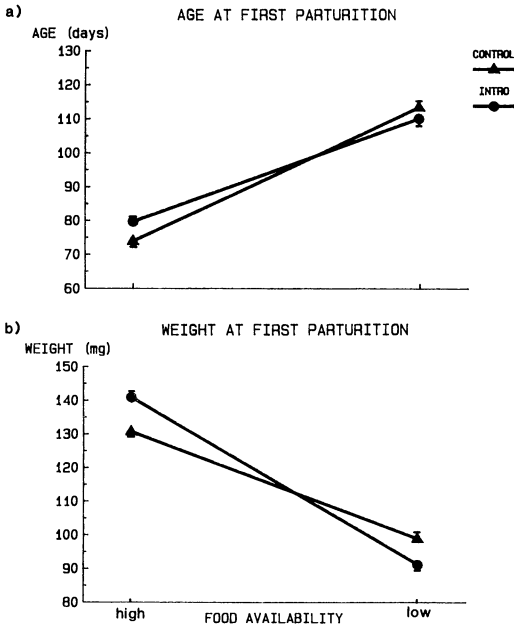
a)

b)

FIG. 4. Female age (±SE) and live weight (±SE) at first parturition in second-generation, laboratory-reared guppies. Weight was measured postpartum. Values are least-square means from a two-way analysis of untransformed data.

this were the case, then the effects of density should be reversed after an interval of time at high population densities. If density and resource availability influence offspring size, then laboratory studies based on later samples should reveal a reversal of this trend.

Further interpretation of these results requires an understanding of all of the factors that may have selected for changes in the life history. The first stage of this introduction experiment exposed the guppies to three potential long-term influences and one transient influence. The long-term effects include changes in predation (from *Crenicichla* to *Rivulus* predation), changes in habitat (such as an increase in canopy cover), and interactions between predation and habitat. The transient influence is the effect of an expanding population. Guppies were introduced at low population densities; population size expanded for an unknown number of generations. Given the multiple factors that might influence life-history evolution, the results to date do not permit any firm conclusions about causality. Furthermore, any conclusions about causality require ad-
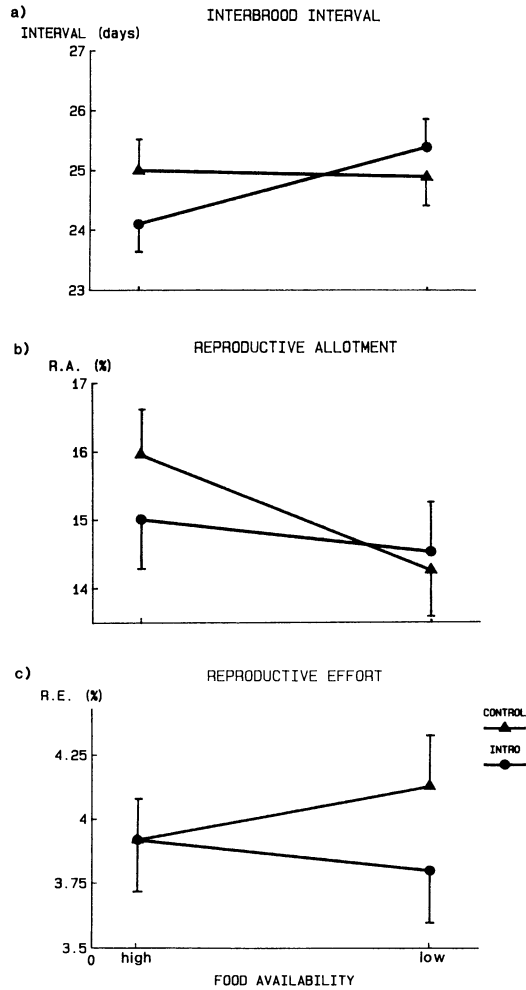
a)

b)

c)

FIG. 5. Life-history variables in second-generation, laboratory-reared females: a) interbrood interval (±SE); b) reproductive allotment (±SE); c) reproductive effort (±SE). Variables are defined in the Materials and Methods. All values are least-square means from the analyses reported in Table 3.

ditional information, such as resource availability, growth rate, and age-specific survival. These other topics are currently being investigated.

Recent papers by Luckinbill and Clare (Luckinbill and Clare, 1985, 1986; Clare and Luckinbill, 1985) present an alternative explanation for the incomplete response to selection reported here for guppies. These authors demonstrated that the response to artificial selection for longevity in *Drosophila melanogaster* was dependent on population density. They were successful in se-
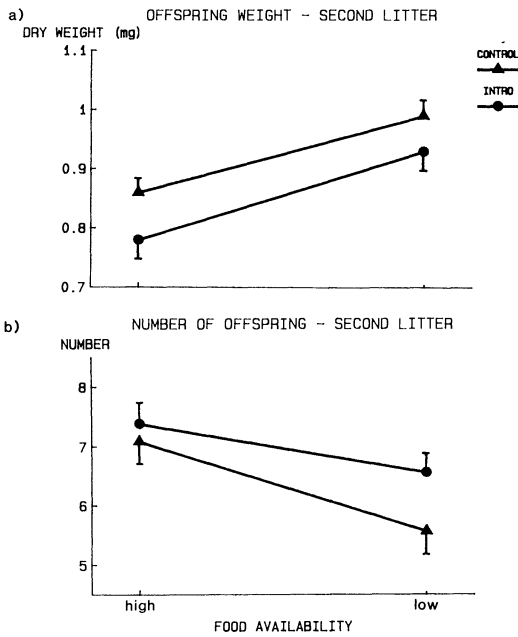
a)

b)

FIG. 6. Life-history variables of second generation, laboratory reared females: a) offspring dry weight (±SE) in the second litter; b) number of offspring (±SE) in the second litter. All values are least-square means from the analyses reported in Table 3.
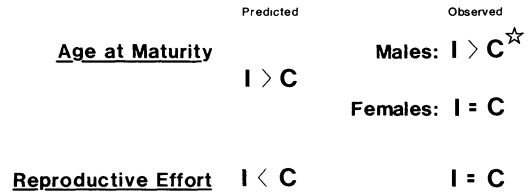

FIG. 7. Summary of the predictions of the age-specific predation hypothesis and our observations on second-generation, laboratory-reared guppies. The star next to the male result indicates that this difference was statistically significant in our laboratory experiment.

lecting for increased or decreased longevity when larvae were reared at high, uncontrolled densities, but not when they were reared at low, controlled densities. These results suggest a genotype × environment interaction, such as the nonexpression of genetic variation for longevity at low densities but significant genetic variation at high densities. Similar interactions, particularly with respect to the transient influence of population density, could have affected the initial outcome of our experiment.

Phenotypic Plasticity.—There were substantial differences in life-history phenotypes of wild-caught versus laboratory-reared guppies, particularly in fecundity and offspring size. In field samples, introduction-site guppies produced fewer, larger offspring than did their downstream-site counterparts (Table 2, Fig. 2). In the laboratory, the introduction-site guppies tended to produce more and smaller offspring (Table 3, Fig. 6). This change between the two sets of observations and the trends seen in the laboratory were completely unexpected and contrary to all of our previous results. For

example, Reznick and Endler (1982) found that guppies in field samples from areas with *Rivulus* produced fewer and larger offspring than did guppies in field samples from *Crenicichla* areas. Reznick (1982*a*, 1982*b*) then found that these differences persisted after two generations in the lab and had a genetic basis.

The response to differences in food availability in the laboratory provides one possible explanation for this plasticity. Increased food availability resulted in a decrease in the age and an increase in size at maturity (males) or first parturition (females), increased fecundity, and decreased offspring size. These effects are identical to those from other experiments with guppies (Reznick, 1982*a*, 1983, unpubl.).

If food was less available in the introduction site than in the downstream control site, then the laboratory results indicate that guppies in the introduction site should produce fewer, larger offspring, which they did. Such differences in resource availability were predicted for *Rivulus* sites in general, because of the higher population densities of guppies and heavier canopy cover. Canopy cover has been associated with reduced productivity in temperate streams (Hawkins et al., 1983). However, other aspects of the life-history patterns of the field-collected guppies are not consistent with this interpretation. For example, the minimum size of wild-caught, reproducing females or mature males is consistently larger in the introduction site. Based on the lower food availability in the introduction site, one would predict that the sizes at maturity would be smaller, relative to downstream-site (Fig. 4). We therefore do not yet have a complete explanation for the changes in

the life history between the field and the laboratory.

Similar reports of the influence of resource availability on life-history variables are readily available for a wide variety of organisms (e.g., Hislop et al., 1978; Schmidt and Gilbert, 1978; Robertson and Salt, 1981; Travis, 1984; Juliano, 1986; Baird et al., 1986). These results, plus our observed shift in life-history patterns between the laboratory and the field, confirm that life histories are highly plastic and that field results alone are an inadequate index of heritable life-history patterns. Any conclusions about trends in life-history evolution that rely on field results alone (e.g., most of the studies cited by Stearns [1977]; Zammuto and Millar, 1985a, 1985b) must therefore be interpreted with caution.

Alternative Explanations. — Five factors possibly complicate our interpretation of these results. The first is the combined influences of predators and resource availability. The proposed second phase of the experiment, in which *Crenicichla* will be introduced over the waterfall, will potentially address this issue. If these predators alter resource availability by reducing guppy population density, then guppy phenotypes should change in a fashion consistent with increased food availability, including increases in growth rate, size at maturity, and fecundity in the introduction site, relative to an upstream control.

A second factor that could cause life-history differences between these populations is genetic drift. We cannot eliminate this alternative, but available evidence argues against it. First, the size of our initial introduction, particularly the number of females (which store sperm and are likely to be multiply inseminated), was sufficient to minimize the influence of drift. Second, our observations five months and 12 months later suggest that the guppy population grew steadily after the introduction.

A third factor could be some form of sampling error in generating our stocks for the laboratory study. The initial sample sizes (23 gravid females per locality) was sufficient for a substantial sampling error to be unlikely. In addition, all but two of these females were equally represented in the second laboratory generation. There was thus

little opportunity for sampling bias or inadvertant selection between the parental and the second laboratory generation.

A fourth possibility concerns the interactions between food availability and locality in male age and size at maturity (Table 3, Fig. 3). These interactions suggest that the expression of life-history differences depends on the level of food availability and that such differences may not be expressed in natural populations; however, the field results for male size at maturity (Table 2, Fig. 1) indicate that these differences are expressed in nature. Furthermore, resource availability and growth rate are being investigated in the field. Our preliminary results suggest that field growth rates are intermediate to the "high" and "low" food treatments in the laboratory, indicating again that the laboratory results realistically represent field performance.

A final possible factor concerns our criteria for concluding that there are genetic differences between the two populations of guppies. Our conclusions are based on differences evident after two generations in a common environment. A more appropriate approach would have included hybridizations between the two localities; however, a power analysis indicated that an experiment that was likely to reveal hybrids as being significantly different from either parental population would have to be prohibitively large. Furthermore, our form of comparison includes maternal effects in the estimated differences between populations. Maternal genetic effects (i.e., the influence of maternal genotype on the phenotype of the offspring, such as reported for offspring size by Reznick, 1982b) are not of concern because they still represent genetic differences between populations; however, maternal environment effects are a potential source of bias. Our design would eliminate any such effect that lasted for one generation, but would not eliminate more persistent maternal effects. Riska et al. (1985) modeled such persistent maternal effects and pointed out that their influence would diminish in subsequent generations, while Falconer (1965) presented an empirical example of such a maternal influence on offspring size. We know of very few empirical examples of such effects, but it is impossible

to say whether the small number is due to the rareness of the phenomenon or to the scarcity of attempts to evaluate it. We consider a bias due to maternal environmental effects to be unlikely, because we carefully randomized the localities at all stages of the rearing. This randomization would both eliminate the single-generation maternal-environmental effects from the wild-caught fish and prevent the introduction of such effects in the laboratory. In addition, the absence of postnatal care in guppies limits the opportunity for transmitting maternal effects. Nevertheless, persistent environmental influences remain a potential source of bias in our comparisons.

Conclusions. —Our results provide partial support for the "age-specific predation" hypothesis by demonstrating the predicted change in age at maturity in males. Lab estimates of female age at first parturition and reproductive effort did not change in the predicted fashion. The age-specific predation hypothesis makes no prediction about fecundity and offspring size, but the changes observed here are contrary to expectations based on previous observations of these fish. The experiment serves as an initial test of the effects of predators but also confounds predation with environmental influences and the potential effects of resource availability. These additional factors could be important in guppy life-history evolution. Furthermore, complete evaluation of the influence of predators requires a direct consideration of age- and size-specific survival in guppies.

These alternatives will be resolved with further investigations. Many of the possible explanations described above imply progressive changes in the life-history patterns of the introduction-site guppies, relative to fish from the downstream-control site. The proposed second phase of introduction (introducing Crenicichla over the barrier waterfall) will provide a critical test of the role of predators, independent of confounding environmental influences, as will replicates of the experiment in different drainages. Ongoing studies of age-structure and survival will provide the necessary link in evaluating how predators select for changes in life histories, if they do. Finally, studies of the quantitative genetics of guppy life histories will help resolve the differences in the responses of males and females to the introduction.

LITERATURE CITED

BAIRD, D. J., L. R. LINTAN, AND R. W. DAVIES. 1986. Life-history evolution and post-reproductive mortality risk. J. Anim. Ecol. 55:295–302.

BARCLAY, H. J., AND P. T. GREGORY. 1981. An experimental test of models predicting life-history characteristics. Amer. Natur. 117:944–961.

———. 1982. An experimental test of life history evolution using Drosophila melanogaster and Hyla regilla. Amer. Natur. 120:26–40.

BOROWSKY, R., AND K. D. KALLMAN. 1976. Patterns of mating in natural populations of Xiphophorus (Pisces: Poeciliidae). I: Xiphophorus maculatus from Belize and Mexico. Evolution 30:693–706.

BOROWSKY, R., AND J. KHOURI. 1976. Patterns of mating in natural populations of Xiphophorus. II. Xiphophorus variatus from Tamaulipas, Mexico. Copeia 1976:727–734.

BOYCE, M. S. 1984. Restitution of r- and K-selection as a model of density-dependent natural selection. Ann. Rev. Ecol. Syst. 15:427–447.

CHARLESWORTH, B. 1980. Evolution in Age-Structured Populations. Cambridge Univ. Press, N.Y.

CLARE, M. J., AND L. S. LUCKINBILL. 1985. The effects of gene-environment interaction on the expression of longevity. Heredity 55:19–29.

DOYLE, R. W., AND W. HUNTE. 1981. Genetic changes in "fitness" and yield of a crustacean population in a controlled environment. J. Exp. Mar. Biol. Ecol. 52:147–156.

DUSSAULT, G. V., AND D. L. KRAMER. 1981. Food and feeding behavior of the guppy, Poecilia reticulata (Pisces:Poeciliidae). Can. J. Zool. 59:684–701.

ENDLER, J. A. 1978. A predator's view of animal color patterns. Evol. Biol. 11:319–364.

———. 1980. Natural selection on color patterns in Poecilia reticulata. Evolution 34:76–91.

————. 1983. Natural and sexual selection on color patterns in Poeciliid fishes. Env. Biol. Fish. 9:173–190.

————. 1986. Natural Selection in the Wild. Princeton University Press, Princeton, NJ.

FALCONER, D. S. 1965. Maternal effects and selection response, pp. 763–774. *In* S. J. Geerts (ed.), Genetics Today. Proc. XI Internat. Congr. Genetics, Vol. 3. Pergamon, Oxford, U.K.

GADGIL, M., AND P. W. BOSSERT. 1970. Life historical consequences of natural selection. Amer. Natur. 104:1–24.

HAIRSTON, N. G., SR. 1980. The experimental test of an analysis of field distributions: Competition in terrestrial salamanders. Ecology 61:817–826.

————. 1986. Species packing in *Desmognathus* salamanders: Experimental demonstration of predation and competition. Amer. Natur. 127:266–291.

HASKINS, C. P., E. F. HASKINS, J. J. A. McLAUGHLIN, AND R. E. HEWITT. 1961. Polymorphism and population structure in *Lebistes reticulata,* a population study, pp. 320–395. *In* W. F. Blair (ed.), Vertebrate Speciation. Univ. Texas Press, Austin.

HAWKINS, C. P., M. L. MURPHY, N. H. ANDERSON, AND M. A. WILZBACH. 1983. Density of fish and salamanders in relation to riparian canopy and physical habitat in streams of the northwestern United States. Can. J. Fish. Aquat. Sci. 40:1173–1185.

HISLOP, J. R. G., A. P. ROBB, AND J. A. GAULD. 1978. Observations on effects of feeding level on growth and reproduction in haddock, *Melonogramnus aeglefinus* (L.) in captivity. J. Fish Biol. 13:85–98.

JULIANO, S. A. 1986. Food limitation of reproduction and survival for populations of *Brachinus* (Coleoptera: Carabidae). Ecology 67:1036–1045.

KOZLOWSKI, J. 1980. Density dependence, the logistic equation, and *r*- and *K*-selection: A critique and an alternative approach. Evol. Theory 5:89–101.

LAW, R. 1979. Optimal life histories under age-specific predation. Amer. Natur. 114:399–417.

LILEY, N. R. 1968. The endocrine control of reproductive behaviour in the female guppy, *Peocilia reticulata* Peters. Anim. Behav. 16:318–331.

LILEY, N. R., AND B. H. SEGHERS. 1975. Factors affecting the morphology and behavior of guppies in Trinidad, pp. 92–118. *In* G. P. Baerends, C. Beer, and A. Manning (eds.), Function and Evolution in Behavior. Oxford Univ. Press, Oxford, U.K.

LUCKINBILL, L. S. 1978. *r*- and *K*-selection in experimental populations of *Escherichia coli.* Science 202:1201–1203.

————. 1979. Selection and the *r/K* continuum in experimental populations of protoza. Amer. Natur. 113:427–437.

————. 1984. An experimental analysis of a life history theory. Ecology 65:1170–1184.

LUCKINBILL, L. S., R. ARKING, M. J. CLARE, W. C. CIROCCO, AND S. A. BUCK. 1984. Selection for delayed senescence in *Drosophila melanogaster.* Evolution 38:996–1003.

LUCKINBILL, L. S., AND M. J. CLARE. 1985. Selection for life span in *Drosophila melanogaster.* Heredity 55:9–18.

————. 1986. A density threshold for the expression

of longevity in *Drosophila melanogaster.* Heredity 56:329–335.

MacARTHUR, R. H., AND E. O. WILSON. 1967. The Theory of Island Biogeography. Princeton Univ. Press, Princeton, NJ.

MICHOD, R. E. 1979. Evolution of life histories in response to age-specific mortality factors. Amer. Natur. 113:531–550.

MUELLER, L. D., AND F. J. AYALA. 1981. Trade-off between *r*-selection and *K*-selection in *Drosophila* populations. Proc. Nat. Acad. Sci. USA 78:1303–1305.

REZNICK, D. N. 1982a. The impact of predation on life history evolution in Trinidadian guppies: The genetic components of observed life history differences. Evolution 36:1236–1250.

————. 1982b. The genetics of offspring size in the guppy (*Poecilia reticulata*). Amer. Natur. 120:181–188.

————. 1983. The structure of guppy life histories: The trade off between growth and reproduction. Ecology 64:862–873.

————. 1985. Costs of reproduction: An evaluation of the empirical evidence. Oikos 44:257–267.

REZNICK, D., AND J. A. ENDLER. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution 36:160–177.

RISKA, B., J. J. RUTLEDGE, AND W. R. ATCHLEY. 1985. Covariance between direct and maternal genetic effects in mice with a model of persistent environmental influences. Genet. Res. 45:287–297.

ROBERTSON, J. R., AND G. W. SALT. 1981. Responses in growth, mortality, and reproduction to variable food levels by the rotifer, *Asplanchna girodi.* Ecology 62:1585–1596.

ROSE, M. R. 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster.* Evolution 38:1004–1010.

ROSE, M. R., AND B. CHARLESWORTH. 1981. Genetics of life history in *Drosophila melanogaster.* II. Exploratory selection experiments. Genetics 97:187–196.

SAS INSTITUTE, INC. 1985. SAS User's Guide: Statistics. SAS Inst., Inc., Cary, NC.

SCHMIDT, J. L., AND D. L. GILBERT. 1978. Big Game of North America: Ecology and Management. Stackpole, Harrisburg, PA.

SEGHERS, B. H. 1973. An analysis of geographic variation in the anti-predator adaptations of the guppy, *Poecilia reticulata.* Ph.D. Diss. Univ. British Columbia, Vancouver, Canada.

————. 1974. Schooling behavior in the guppy (*Poecilia reticulata*): An evolutionary response to predation. Evolution 28:486–489.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry, 2nd Ed. Freeman, San Francisco, CA.

SOLBRIG, O. T., AND B. B. SIMPSON. 1977. A garden experiment on competition between biotypes of the common dandelion (*Taraxacum officinale*). J. Ecol. 65:427–430.

STEARNS, S. C. 1976. Life history tactics: A review of the ideas. Quart. Rev. Biol. 51:3–47.

————. 1977. The evolution of life history tactics: A critique of the theory and a review of the data. Ann. Rev. Ecol. Syst. 8:145–171.

TAYLOR, C. R., AND C. CONDRA. 1980. r- and K-selection in *Drosophila pseudoobscura*. Evolution 34: 1183–1193.

TRAVIS, J. 1984. Anuran size at metamorphosis: Experimental test of a model based on intraspecific competition. Ecology 65:1155–1160.

TURNER, C. L. 1941. Morphogenesis of the gonapodium in *Gambusia affinis affinis*. J. Morphol. 69: 161–185.

ZAMMUTO, R. M., AND J. S. MILLAR. 1985a. A consideration of bet-hedging in *Spermophilus columbianus*. J. Mammal. 66:652–660.

———. 1985b. Environmental predictability, variability, and *Spermophilus columbianus* life history over an elevational gradient. Ecology 66:1784–1794.

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