

Divergent life histories among populations of the fish *Brachyrhaphis rhabdophora*: detecting putative agents of selection by candidate model analysis

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Studies of natural selection in the wild almost always begin by examining patterns of association between phenotypic adaptations and environmental factors thought to shape evolutionary change. Unfortunately, many studies pay little attention to the effects of model selection on the evolutionary inferences drawn from such correlative data. In this study, I employed a candidate model analysis to examine four potential causes of life-history evolution in the livebearing fish *Brachyrhaphis rhabdophora*. Combining factor analysis with path analysis, I constructed a nested set of 17 models that represent the hypothetical effects of four putative selective agents (mortality, density, resource availability, and habitat stability) on life-history evolution in this species. These models represent both direct and indirect effects of selection on the life history. Using the Akaike Information Criterion to distinguish among models, I found that simple models that contained only single selective agents most parsimoniously explained life-history divergence among 27 *B. rhabdophora* populations. Surprisingly, the four putative selective agents could not be distinguished, suggesting that the selective environment could be composed of a single selective agent confounded with other environmental factors, or could be composed of a suite of environmental factors that act in concert to shape the life history. In addition, comparisons among more complex models indicated that direct effects of selective agents appear to have primacy over combinations of indirect selective interactions in explaining intraspecific variation in *B. rhabdophora* life histories.

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Comparative studies have long been used to identify agents of natural selection, particularly those agents responsible for the evolutionary diversification of life histories (Brooks and McLennan 1991, Roff 1992). Intraspecific comparisons among populations have proven especially useful for examining selection in the wild. Unlike higher taxonomic units, populations of the same species tend to be more recently separated and more likely to reside in the selective environments where differences evolved (Foster 1999). Although the comparative approach cannot demonstrate

selection unequivocally, and cannot be used to directly infer causal relationships, close correlation between life histories and potential selective agents can point to probable causes of life-history divergence that can then be tested using other methods (Endler 1986).

Despite the widespread use of correlative data as a means of exploring life-history variation, little attention has been paid to the effects of model selection on biological inferences drawn from such data (Hilborn and Mangel 1997, Burnham and Anderson

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1998, Anderson et al. 2000). For example, models that include all possible combinations of environmental factors are often overfitted (i.e., lack generalization) and can produce spurious correlations that are positively misleading. In contrast, models with too few parameters might fail to identify potential selective agents, or could fail to detect interactions between two or more selective agents that might act in concert to shape the life history. To avoid such pitfalls, Burnham and Anderson (1998) advocate formulating an a priori set of candidate models that reflect biologically meaningful hypotheses and that can be individually evaluated for fit to the data collected. A strength of this approach is that recent advances from information theory can be used to select a “best approximating” model from a set of candidate models. Once identified, a best fitting model, or a set of best fitting models, can be used to infer probable agents of selection.

In this study, I construct a set of nested models using path analysis to explore divergent life-history strategies observed in the livebearing freshwater fish *Brachyrhaphis rhabdophora* (Poeciliidae). Previous work has shown that predation environment clearly predicts phenotypic divergence in life histories among *B. rhabdophora* populations (Johnson and Belk 2001) and that these differences have a genetic basis (Johnson 2001). Here, I decompose predation environment into its constituent parts in an attempt to identify specific environmental factors that could have shaped such divergence. I model four hypothetical causes of life-history variation: (1) extrinsic mortality (Law 1979, Kozłowski and Uchmanski 1987, Abrams and Rowe 1996); (2) density dependence (Boyce 1984, Elgar and Catterall 1989, Mylius and Diekmann 1995); (3) resource availability (Twombly et al. 1998, Reznick et al. 2000, Shanley and Kirkwood 2000); and (4) habitat stability (Slatkin 1974, Phillippi and Seger 1989, Benton and Grant 1999). By design, these models are not exhaustive, but rather reflect a set of plausible hypotheses taken from life-history theory to understand life-history divergence in this system. I compare these candidate models to evaluate the extent to which the data support competing hypotheses. By using this model-testing approach, I move beyond comparative studies that assume a single factor is responsible for life-history divergence (i.e., studies that ignore the possibility of multiple selective agents and interactions between such agents). I also avoid problems of ‘overfitting’ that can result from using multiple regression to predict life-history traits from a large number of abiotic and biotic variables (Crespi and Bookstein 1989). By combining model testing with path analysis, I present a general framework for conducting comparative research where correlative data are the primary means of biological inference.

Methods

Study system

Brachyrhaphis rhabdophora is endemic to continental northwestern Costa Rica and is widely distributed throughout a variety freshwater stream habitats (Bussing 1987). Populations exist under a broad range of environmental conditions that include site-specific differences in fish density, stream productivity, physical habitat characteristics, and fish community structure. In some locations *B. rhabdophora* coexists with natural predators including the cichlid fish *Cichlasoma dovii* and the catfish *Rhamdia guatemalensis*; in other locations *B. rhabdophora* is the only fish species present. Such variation in environments provides a range of selective conditions under which populations might evolve local adaptations.

Marked life-history variation has been documented among *B. rhabdophora* populations (Reznick et al. 1993, Johnson and Belk 2001). Reproduction is accomplished by internal fertilization and females give birth to fully developed live young approximately every 4 weeks throughout the year. Within these constraints, populations show remarkable differences in offspring size, fecundity, reproductive allocation, size at maturity of females, and size at maturity of males (Reznick et al. 1993). This variation is widespread throughout the range of the species and occurs across geographically complex environments, a pattern well suited for identifying putative selective agents using correlative data (Endler 1986).

Sampling methods and environmental characteristics

I sampled *B. rhabdophora* in three successive years (1996–1998) at the end of the dry season (April/May)

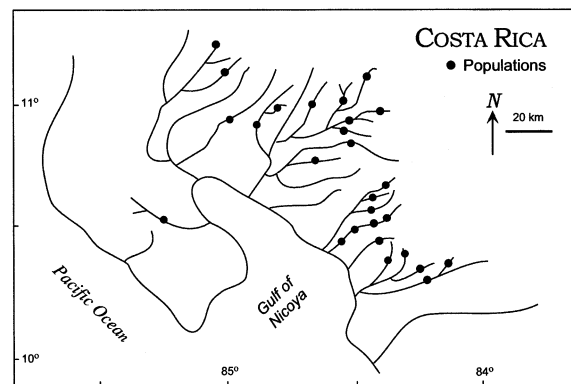


Fig. 1. Distribution map of the fish *B. rhabdophora* in northwestern Costa Rica identifying collecting sites for populations included in this study. Specific map coordinates are available upon request.

and in one year (1999) at the end of the wet season (January). In total, I collected from 27 different sites throughout northwestern Costa Rica (Fig. 1), 12 of which were represented during all four collecting periods. This sampling scheme was designed to explore life-history variation over time and across seasons. Fish were collected with a hand-held seine (1.3 m × 5 m; 8 mm mesh size). Approximately 200 individuals were taken from each site, a sample size that ensured adequate representation of both mature and immature males and females, but was only a small fraction of the local population. All fish were preserved in ethyl alcohol in the field and were transported to the laboratory for further analysis.

For each collecting site, I quantified a set of environmental parameters – these were used to evaluate the relationship between potential selective agents and *B. rhabdophora* life histories (as modeled below). I classified each population by predation level (0 or 1), determined by the presence or absence of the dominant piscivorous fishes *C. dovii* and *R. guatamalensis*. Relative density of *B. rhabdophora* was estimated by calculating the average number of fish collected per seining attempt, a value I found to be positively correlated with actual density (fish/m³; $r = 0.56$, $n = 17$, $p = 0.02$). Finally, I measured stream width, flow rate, percent canopy cover, and stream gradient at each site. Stream width and flow rate were measured directly using a tape measure, and by timing a floating object, respectively. Canopy cover was measured with a hand-held densiometer, and stream gradient was calculated from topographic maps.

Characterization of the life history

I measured five life-history traits for each population. Traits evaluated were size at maturity for (1) males and (2) females, (3) reproductive allotment by females, (4) number of offspring, and (5) size of offspring. All life-history data were collected in the laboratory from the alcohol-preserved specimens using methods described in Reznick et al. (1993). In brief, because males cease to grow upon maturation, I estimated size at maturity as the average standard length of adult males; mature males were distinguished by complete development of the modified anal fin (gonopodium) used in sperm transfer (as in other Poeciliid fishes; Turner 1941, Constanz 1989). The minimum size class of reproducing females, an estimate of the age at which females first reproduce, was scored as the 2-mm size class in which at least half of the individuals contained developing embryos (defined here as stage 3 or greater following Haynes (1995)). Reproductive allotment was calculated as the dry mass of a single brood of offspring. Number of offspring equaled the total number of individuals in the developing brood. Offspring size equaled the aver-

age dry weight of developing offspring. Only females with developing embryos were included in the estimates of reproductive allotment, number of offspring, and offspring size.

To generate comparable estimates among populations for reproductive allotment, number of offspring, and size of offspring, I adjusted these life-history traits by analysis of covariance (ANCOVA) using the following covariates. Reproductive allotment (brood dry mass) and offspring size were adjusted for maternal dry mass and brood developmental stage. Number of offspring was adjusted only for maternal dry mass. The covariates were statistically significant in all cases ($p < 0.05$). In most cases there was not a significant interaction between population and the covariate (i.e., slopes did not differ); however, when such interactions did occur, the regression lines typically crossed beyond the bounds of biologically meaningful sizes and thus did not qualitatively alter the adjusted life-history trait (see Johnson and Belk 2001). Hence, my comparisons between populations are based upon adjusted least square means generated by the ANCOVA models. To satisfy assumptions of the ANCOVA, all size measurements were log₁₀ transformed and counts were square-root transformed.

Life-history theory is underpinned by the assumption that life histories evolve as an integrated suite of traits and that variation among these traits is largely constrained by genetic trade-offs (Fisher 1930, Reznick 1985, de Jong and Van Noordwijk 1992). This assumption is important in this study as it affects whether or not the life history can be evaluated as a collective strategy or if each life-history trait should be considered separately. I assessed the phenotypic correlation structure among traits using principal components analysis with principal components extracted from correlation matrices (as in Strauss 1990). I recognize that phenotypic covariance among life-history traits does not necessarily reflect the underlying genetic covariance structure and that any conclusions about life-history trade-offs will ultimately require investigation of the latter (Roff 1992). Nonetheless, I found that for each of the four collecting periods (three dry seasons and one wet season), the five life-history traits behaved as a suite of intercorrelated variables that could be collapsed to two principal components that in each case explained > 80% of the variation in the data (Table 1). Given this, I modeled the life history of *B. rhabdophora* as two orthogonal unmeasured factors that could be predicted by the five measured life-history traits (Sokal and Rohlf 1995: 680).

Characterization of selective agents

I evaluated four potential selective agents: (1) extrinsic mortality; (2) density dependence; (3) resource

Table 1. Results of principal components analyses evaluating five life-history traits in *B. rhabdophora* examined over four collecting periods (1996–1998 = dry season; 1999 = wet season). Listed under PC1 and PC2 are the component loadings (i.e., coefficients of the standardized factors scores under a varimax rotation) for each life-history trait on the first two principal components, evaluated by year. The number of sites evaluated for each year is given in parentheses.

Life-history trait	Year:	1996 DRY (n = 21)		1997 DRY (n = 14)		1998 DRY (n = 16)		1999 WET (n = 19)	
		PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Male size at maturity		0.89	0.06	0.92	0.01	0.83	0.28	-0.42	0.84
Female size at maturity		0.83	-0.28	0.79	0.07	0.76	0.19	0.09	0.94
Brood mass		0.04	0.98	0.23	0.94	-0.91	0.02	0.93	0.04
Number of offspring		-0.42	0.89	-0.39	0.91	-0.75	-0.55	0.91	0.36
Size of offspring		0.82	-0.19	0.76	-0.44	0.12	0.99	-0.57	0.64
Variance (%)		46	38	51	33	53	28	44	42
Cumulative (%)		46	84	51	84	53	81	44	86

availability; and (4) habitat stability. I assume in this study that these agents are unmeasured factors that are correlated with measurable environmental characteristics (as advocated by Crespi and Bookstein 1989). These characteristics are considered to contain biological information only insofar as they are correlated with the underlying selective agent. I modeled these correlations by path analysis, a method developed by Sewall Wright (1921) to estimate correlation between observed characters and unobserved factors (Li 1975). Hence, my characterization of the selective agents represents an attempt to identify environmental parameters that are directly correlated with the unmeasured (and in some cases, unmeasurable) true selective agents. In addition, path analysis allowed me to estimate the indirect effects of one putative selective agent upon another (e.g., Shine 1996).

Selective agents were characterized as follows. (1) Extrinsic mortality was estimated by the parameter 'predation level' and (2) density dependence was estimated by the parameter 'relative density'. (3) Resource availability was estimated by the parameter 'canopy cover'. I used canopy cover as an indicator of primary productivity which here is assumed to be related to resource abundance. (4) Habitat stability was estimated by the parameters 'stream gradient', 'stream width', and 'flow rate'; in choosing these parameters, I assumed that flooding caused by daily and seasonal rains reflects the major component of habitat instability. Correlation structure among these measured variables showed that environments with predators generally had lower *B. rhabdophora* densities, wider and lower-gradient streams, and reduced canopy cover relative to predator-free environments (Table 2). However, it is important to note that none of the measured environmental parameters included here is expected to perfectly describe the underlying selective agent or to predict the life history – that is, I do not assume that these measures are direct surrogates for selection. Rather, I expect that the measured parameters are correlated with the true selective agents. It is

precisely the inability of measured traits to predict the life history that motivates me to model the putative selective agents as unmeasured factors. A statistical advantage of modeling these relationships by path analysis is that increasing the number of measured traits increases the ability to describe the underlying selective agents. Hence, unlike multiple regression, the method is relatively insensitive to the addition or deletion of measured traits (Kingsolver and Schemske 1991).

Generating candidate models, path analysis, and model selection

I generated an a priori set of 17 candidate models (path diagrams; Table 3) that reflect hypothetical relationships between four putative selective agents and the *B. rhabdophora* life history (depicted in Fig. 2 as a single global model). These structural models represent an attempt to describe both direct and indirect effects of selective factors on life-history variation. By design, not all combinations of factors, or all possible interactions between factors, are evaluated. I focused instead on those paths and combinations of paths that represent plausible hypotheses taken from life-history theory (reviewed in Roff 1992) to understand life-history divergence in this system (Table 3).

Path analysis is a system of portraying and analyzing models of linear causation (Sokal and Rohlf 1995). Multiple regression, factor analysis, and other systems of linear relationships can be expressed as a path analysis (Li 1975). In this study, I combined factor analysis (used to estimate the unmeasured life history and selective agents) with multiple regression to evaluate correlation between selective agents and the life history. The first step in a path analysis is to construct the path diagram – a model of hypothetical relationships between variables. I generated a global model path diagram that depicts direct and indirect effects of four putative selective agents on the life history of

Table 2. Coefficients for pairwise correlations among environmental variables used to characterize the four putative selective agents (see text for details). Environmental variables were measured over four collecting periods including three dry-season samples (1996–1998) and one wet-season sample (1999).

Environmental variable	Predation	Density	Percent canopy	Stream gradient	Stream width
1996 ^a and 1997 ^b					
Predation	–	–0.36	0.04	–0.61	0.68
Density	–0.49	–	–0.18	0.09	–0.28
Percent canopy	–0.51	–0.19	–	–0.27	–0.01
Stream gradient	–0.69	–0.42	0.38	–	–0.43
Stream width	0.61	–0.23	–0.45	–0.37	–
1998 ^a and 1999 ^b					
Predation	–	–0.11	–0.07	–0.46	0.57
Density	0.04	–	–0.27	0.77	–0.46
Percent canopy	–0.35	0.22	–	–0.08	0.15
Stream gradient	–0.71	0.19	0.26	–	–0.39
Stream width	0.51	–0.21	–0.20	–0.54	–

^a Above the diagonal. ^b Below the diagonal.

B. rhabdophora (Fig. 2). I ran the path analysis for the 17 candidate models over the four separate collecting periods using the software Amos 4.0 (Arbuckle and Wothke 1999). Path analyses were based on correlation matrices of the measured environmental parameters and life-history traits described above.

I evaluated the fit of each model to the data using the maximum likelihood model-fitting routine in Amos 4.0 (Arbuckle and Wothke 1999). This routine generates an Akaike Information Criteria (AIC) value for each model represented in the path diagram. To adjust for bias due to small sample sizes relative to the number of parameters evaluated in the candidate models, I used the AIC_c adjustment as recommended by Burnham and Anderson (1998: 51). In brief, the AIC_c value describes the amount of information explained by a particular model relative to the total information contained in the data; the smaller the score, the better the model fits the observed data (Akaike 1983, Browne and Cudeck 1993). As a rule of thumb, only models with AIC_c scores that differ by >2 provide distinguishable levels of support (Burnham and Anderson 1998: 323) and I used this guideline to draw inferences about which models provided better fits. The model fitting approach employed here has the property that the AIC_c value decreases when additional parameters are added to a model only if the improved fit (i.e., reduced discrepancy between the candidate model and the “true” model) is sufficient to offset the “penalty” of including additional parameters – otherwise, the AIC_c increases. Hence, this application of parsimony gives some guidance as to the number and combination of parameters that provide the best fit. I used AIC_c estimates to rank models evaluated in this study and from these candidate models inferred putative selective agents that best explain the observed life-history differences among *B. rhabdophora* populations.

Results

Model complexity

In evaluating *B. rhabdophora* life histories, I found that simpler models (i.e., those with fewer parameters) had distinguishably lower AIC_c scores than more complex models, indicating that the inclusion of additional parameters typically did not improve the amount of information parsimoniously described by the model (Table 4). This trend was particularly evident when considering the number of putative selective agents included in a model; in all cases, models that included fewer selective agents (ranging from 1 to 4) were superior to models that included a greater number of selective agents. In fact, models that included only a single putative selective agent at a time did a markedly better job of explaining *B. rhabdophora* life histories than all other models in the set. This pattern held up across all

Table 3. Putative selective agents evaluated in the structural model of the path analysis defined by their hypothetical effects on the life history (LH). Path numbers correspond to those diagrammed in Fig. 2.

Hypothesis	Path
Direct effects	
Resource availability shapes LH	1
Density shapes LH	2
Mortality shapes LH	3
Habitat stability shapes LH	4
Indirect effects	
Density determines resource availability	5
Density influences mortality	6*
Mortality alters density	6*
Habitat stability alters density	7
Unknown effects	
Variation in the LH not explained by the combination of other effects	8

* These hypotheses share a correlative path and therefore cannot be distinguished in the model.

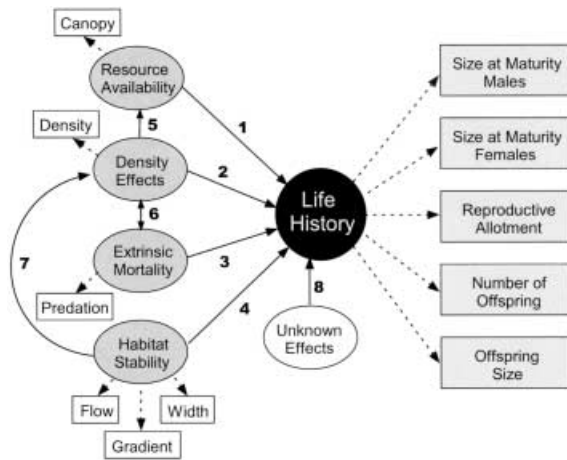


Fig. 2. A global model path diagram that depicts direct and indirect effects of four putative selective agents on the life history of *B. rhabdophora*. Selective agents (light-shaded circles) are unmeasured underlying factors that are estimated from a set of measured environmental parameters (unshaded boxes). The life history (dark-shaded circle) is also evaluated as a pair of unmeasured underlying factors generated from five measured life-history traits (shaded boxes). Residual variation – that not explained by the combination of selective agents – is depicted as an unmeasured factor (unshaded circle). Numbered arrows indicate the regressions of the putative selective agents on the life history (direct effects) and on each other (indirect effects); these numbers correspond to hypothetical effects described in Table 1. Dashed arrows projecting from selective agents are the factor loadings of the measured environmental traits and were used to determine the underlying selective agents. Dashed arrows projecting from the factor Life History are the factor loadings of the measured life-history traits and were used to determine the underlying *B. rhabdophora* life history.

four sampling periods indicating a consistency across years and between wet and dry seasons.

Detecting putative selective agents

Of the four putative selective agents evaluated in this study (Fig. 2, Table 3), no single factor explained life-history variation in *B. rhabdophora* better than any other factor. AIC_c scores for all single-factor models (i.e., one selective agent considered at a time) differed by less than two units, suggesting that the four selective agents were indistinguishable in their ability to explain life-history differences among populations. This was further demonstrated by comparing the three sets of two-factor models – from these sets, the five best models (AIC_c values ranging from 28.95 to 30.75) were spread evenly among the different combinations of the four selective agents evaluated. Thus, models that included density, resource availability, mortality, or habitat stability were similar in their ability to explain variation in the life-history data.

Direct versus indirect effects

In almost all cases, direct effects of putative selective agents were more strongly supported than indirect effects (Table 4). Comparisons made among distinguishable models at comparable nesting levels within years showed that in 8 of 11 cases, the model with the significantly lower AIC_c score (difference of at least two AIC_c units) included only direct effects. Hence, interactions between density and resource availability (path 5 in Fig. 2), between mortality and density (path 6), and between habitat stability and density (path 7), all explain less variation in the life history than do the direct effects of these variables on the life history. One exception to this general trend was the case where habitat stability predicted density (path 7), and density in turn, predicted the life history (path 2), a pattern found in the 1997 and 1998 collections.

Discussion

Identifying the best model

The primary objective of this study was to test a set of a priori hypotheses taken from life-history theory to identify likely causes of life-history divergence among *B. rhabdophora* populations. These hypotheses were modeled as a nested set of path analyses that included various combinations of four putative selective agents characterized by both direct and indirect effects on the life history (Table 3). I used Akaike's Information Criteria (AIC_c) to identify those models (i.e., hypotheses) that were most consistent with the data. I found that in each of the four sampling years, the set of models that included only a single selective agent consistently outperformed more complex models in their ability to parsimoniously explain variation in the *B. rhabdophora* life history. This trend persisted across the nested hierarchy such that models that included fewer putative selective agents, regardless of how these agents were related in the path diagram, always provided a better fit than models that included more selective agents.

In terms of the model selection criteria, this trend suggests that the inclusion of additional putative selective agents in a hypothesis does not explain additional information in the observed life-history data, or that the additional information explained is insufficient to offset the parsimony "penalty" of including additional parameters. What biological conditions would lead to such a pattern? One possibility is that estimates of the putative selective agents are highly correlated such that each variable explains essentially the same information in the life history. In other words, if the four potential selective agents are to some degree confounded (e.g., if predators decrease prey density, increase per capita

resource availability, and occur only in more productive, stable habitats), then any single factor is equally likely to explain life-history divergence among populations. Under such conditions, the most parsimonious way to explain differences among populations is to consider only single-factor models.

Distinguishing among putative selective agents

Although it is clear that single-factor models are superior to multifactor models, I found no evidence that any single putative selective agent was superior to the other three. In fact, in each of the four years, the best model (i.e., lowest AIC_c) included a different selective agent (Table 4). However, in none of these years could the single-parameter models be distinguished from one another (i.e., no AIC_c differences > 2).

An inability to distinguish among putative selective agents suggests two possibilities. First, one single selective agent could be driving evolutionary differences among populations and other environmental factors could simply be confounded with this agent. For example, in previous work I have shown that divergent life-history evolution among *B. rhabdophora* populations is consistent with differences in age-specific mortality regimes: fish from sites that co-occurred with predators matured at smaller sizes and produced larger broods of smaller offspring relative to fish from predator-free environments (Johnson 2001, Johnson and Belk 2001). Hence, it is possible that differences in predator-mediated mortality have primacy in shaping the life history, but are coincidentally confounded with density, resource

availability, and habitat type. Alternatively, two or more of these selective agents might act in concert to shape the life history (e.g., Abrams and Rowe 1996). Ultimately, experimental work will be necessary to distinguish among these alternatives (sensu Endler 1986). However, the model selection approach employed here shows that none of the putative selective agents evaluated in this study can be discarded based on correlative data alone.

Relationships between putative selective agents and *B. rhabdophora* life histories were consistent over time (Table 4) and across models of varying complexity (as exemplified in Fig. 3). Extrinsic mortality showed a strong negative association with size at maturity and offspring size (traits with high loadings on life-history factor 1), and a positive association with brood mass and number of offspring (traits with high loadings on life-history factor 2). Density and habitat stability were both positively associated with size at maturity and offspring size, but negatively associated with reproductive effort. In contrast, resource availability was positively associated with both of the life-history factors, suggesting that increased food resulted in increases in offspring size, size at maturity, and reproductive output (Reznick et al. 2000). Hence, although no single selective agent was superior to other agents, I found the associations between selective agents and life-history traits to be quite stable.

Primacy of direct effects

My results suggest that interactions between putative selective agents are less important in shaping the *B. rhabdophora* life history than are direct selective effects.

Table 4. An a priori set of 17 candidate models that reflect biologically meaningful hypotheses to explain life-history variation among populations of the fish *B. rhabdophora*. Selective agents are abbreviated as follows: R = resource availability; D = density effects; M = extrinsic mortality; H = habitat stability. Models are defined by their constituent paths as shown in Fig. 2. Sample sizes (*n*) show the number of populations evaluated for fit to each candidate model in each respective year.

Selective agents	Model (defined by paths)	AIC _c 1996 <i>n</i> = 21	AIC _c 1997 <i>n</i> = 14	AIC _c 1998 <i>n</i> = 16	AIC _c 1999 <i>n</i> = 19
R	1-8	20.24	18.53	18.89	19.88
D	2-8	20.42	18.40	19.13	19.72
M	3-8	20.93	18.28	18.88	22.05
H	4-8	20.70	18.21	19.59	20.15
R-D	1-2-8	33.12	29.62	31.52	32.53
R-D	1-5-8	32.59	32.58	32.34	29.75
R-D	1-2-5-8	36.49	32.76	34.18	35.58
D-M	2-3-8	36.09	32.78	30.75	34.45
D-M	2-6-8	38.74	32.36	34.03	46.56
D-M	2-3-6-8	37.43	32.85	34.33	38.36
D-H	2-4-8	34.14	31.26	42.21	32.68
D-H	2-7-8	38.98	28.95	29.63	38.40
D-H	2-4-7-8	37.68	32.87	34.39	35.63
R-D-M	1-2-3-8	48.63	53.29	43.30	50.01
R-D-M	1-2-3-6-8	49.96	53.36	46.89	53.92
R-D-M	1-2-3-5-6-8	53.33	56.50	49.55	56.98
R-D-M-H	1-2-3-4-5-6-7-8	78.00	84.06	71.80	84.28

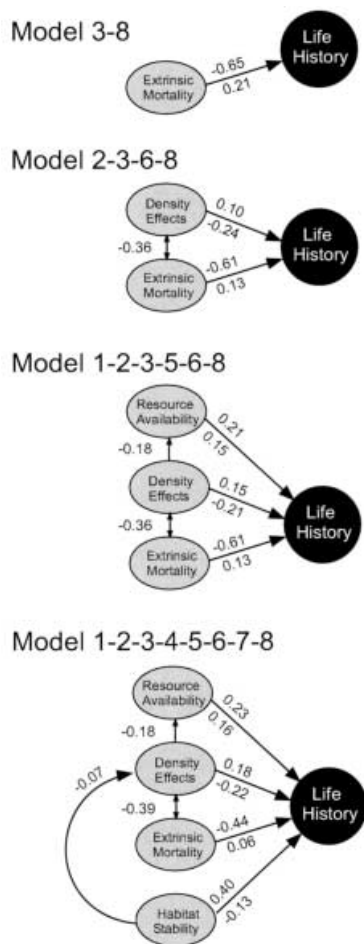


Fig. 3. Path diagrams for a subset of four models (based on 1996 dry season data) used to illustrate associations between putative selective agents and the life history as a function of model complexity. The four models presented here increase in complexity ranging from a model with a single selective agent (top panel) to a full model that includes all four putative selective agents and interactions among these agents (bottom panel). Values associated with arrows are path coefficients depicting the direction and strength of association between variables. Paths from the putative selective agents (light shaded circles) to the life history (dark shaded circle) have two coefficients corresponding to the two orthogonal factors derived from the five measured life-history traits (see text for details). In these cases, the path coefficient above the line predicts size at maturity and offspring size (factor 1), and the coefficient below the line predicts reproductive allotment and number of offspring (factor 2).

In most cases, models that included only direct effects provided a distinguishably better fit to the data than counterpart models that included both direct and indirect effects, with one possible exception. There appears to be some evidence that habitat stability has a greater indirect effect on the life history via density than it has on the life history itself. Fish from environments characterized by smaller, shallower stream channels tend to have higher densities. Hence, the physical structure of the stream, modeled above as a predictor of habitat

stability, appears to indirectly contribute to life-history divergence by altering *B. rhabdophora* densities.

Indirect effects are known to be important in ecological and evolutionary interactions across a wide variety of taxa (e.g., Brown et al. 1986, Brönmark et al. 1992, Paine 1992). In fact, indirect effects in livebearing fishes may be quite common. Consider three examples. First, life-history differences in Trinidadian guppies, once ascribed primarily to variation in mortality (Reznick et al. 1990, Strauss 1990), appear also to be influenced by predator-mediated changes in density and per-capita resource availability (Grether et al. 2001, Reznick et al. 2001). Second, Stearns (1983a, b) found that mosquitofish (*Gambusia affinis*) in fluctuating environments experience higher overall mortality rates than their counterparts in stable environments. Such habitat instability was associated with divergent life histories, but the underlying cause of life-history divergence could have been due to differences in mortality, density, or some combination of these factors (Stearns 1983a). Finally, body size evolution in sailfin mollies (*Poecilia latipinna*) appears to be shaped by complex interactions between predation and abiotic stress, modulated by stochastic variation in temperature and salinity across time (Trexler et al. 1992). Yet, despite the seeming ubiquity of such indirect interactions among selective agents in other livebearing fishes, direct effects by putative selective agents appear to be more important in *B. rhabdophora* than indirect effects.

Caveats and limitations of the approach

Characterizing a selective regime using point estimates of environmental data and phenotypic measures of life histories presents two unique problems. The first problem is that phenotypic data alone tell us little about the genetic basis for life-history differences among populations. Hence, experimental work targeted at measuring the genetic component of phenotypic variation is essential to infer that natural selection has been operating (Endler 1986). In *B. rhabdophora*, I used common-environment experiments to show that differences in male life-history phenotypes observed in the field are genetically based (Johnson 2001); preliminary data from females support a similar conclusion (Johnson unpubl.).

The second problem with the correlative approach is that environmental parameters are typically measured as point estimates over short ecological time scales, whereas adaptive evolution reflects changes over long-term selective conditions. Hence, care should be taken to quantify putative selective agents over a temporal scale that captures a meaningful range of environmental variability, both for ecological parameters and for the phenotypic traits of interest. Here, this required comparing populations over multiple years and in both wet and dry seasons. By comparing life-history traits over this time period, I previously described a plastic

shift in reproductive effort between wet and dry season samples (Johnson and Belk 2001). However, the consistent association across sampling periods between life histories and putative selective agents evaluated in this study (Table 4) suggests that the selective environment – as modeled here – is stable, despite possible short-term variation in ecological conditions.

Conclusions

Correlative data have long been used in hopes of identifying meaningful lines of experimental inquiry, especially in research programs that attempt to identify natural selection in the wild. The model-testing approach employed here suggests that although divergent life-history evolution in *B. rhabdophora* is well predicted by “predation environment” (Johnson and Belk 2001), there is considerable overlap in the potential selective agents that might compose this environment. Hence, the results of this study indicate that experimental work in this system targeted at examining the independent and interactive effects of mortality, density, habitat stability, and resource availability, will provide the most compelling line of inquiry for future research.

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