

Article

Life History Divergence in Livebearing Fishes in Response to Predation: Is There a Microevolution to Macroevolution Barrier?

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Received: 10 April 2020; Accepted: 3 May 2020; Published: 5 May 2020



Abstract: A central problem in evolutionary biology is to determine whether adaptive phenotypic variation within species (microevolution) ultimately gives rise to new species (macroevolution). Predation environment can select for trait divergence among populations within species. The implied hypothesis is that the selection resulting from predation environment that creates population divergence within species would continue across the speciation boundary such that patterns of divergence after speciation would be a magnified accumulation of the trait variation observed before speciation. In this paper, we test for congruence in the mechanisms of microevolution and macroevolution by comparing the patterns of life history divergence among three closely related species of the livebearer genus *Brachyraphis* (Poeciliidae), namely *B. rhabdophora*, *B. roseni*, and *B. terrabensis*. Within *B. rhabdophora*, populations occur in either predator or predator-free environments, and have been considered to be at a nascent stage of speciation. Sister species *B. roseni* and *B. terrabensis* are segregated into predator and predator-free environments, respectively, and represent a post-speciation comparison. Male and female size at maturity, clutch size, and offspring size (and to a lesser extent reproductive allocation) all diverged according to predation environment and differences were amplified through evolutionary time, i.e., across the speciation boundary. Variation observed among nascent species differentiated by predation environment is a good predictor of variation among established species differentiated by predation environment. We found no evidence for different processes or different levels of selection acting across the speciation boundary, suggesting that macroevolution in these species can be understood as an accumulation of micro-evolutionary changes.

Keywords: microevolution; speciation; macroevolution; life history; Poeciliidae; predation environment

1. Introduction

An unresolved issue of the modern evolutionary synthesis is whether or not evolutionary diversification prior to speciation, i.e., early in the speciation continuum, is fundamentally different from evolutionary divergence after new species have formed, i.e., at later stages of the speciation continuum [1–4]. This potential distinction between ‘micro’ and ‘macro’-evolution has engendered a long-running argument in biology and the philosophy of science (see recent reviews in [1–5]), and provided a line of demarcation for some opponents of the theory of evolution who concede microevolution but reject speciation. Yet, early architects of modern synthesis generally drew no distinction between mechanisms of micro and macro-evolutionary change (reviewed in [4]).

Those who view macroevolution as distinct in some fundamental way argue that processes that govern divergence among species are based on the selection of emergent properties of species (or higher taxonomic levels), and thus are different from processes that cause variation among populations and individuals within species [2,6]. Unfortunately, although recent studies have begun to address this important issue (e.g., [7,8]), we still have relatively few studies that explore evolution on both sides of the speciation boundary, particularly where similar forces of selection are at play. Many recent efforts to understand variation throughout the speciation process have focused on the initiation of the speciation process by evaluating patterns of divergence in so called nascent or incipient species [9–18]. Although these studies have informed trait divergence in early stages of speciation, they do little to answer the question of whether there is a fundamental distinction between micro- and macro-evolutionary processes. Similarly, studies of variation among species and higher taxonomic groups are largely based on describing patterns from the fossil record and inferring mechanisms of macro-evolutionary change [19–21]. However, many traits relevant to species diversification are not preserved in the fossil record. Thus, these studies also provide a poor comparison to evolutionary processes occurring prior to speciation. One way to bridge this gap and test the idea of similar evolutionary processes operating on both sides of the species boundary is to compare evolutionary diversification patterns within species prior to speciation to evolutionary change after speciation has occurred (see, for example [7,8]). If patterns of divergence among populations within a species (i.e., nascent species) are in similar directions and magnified among more divergent species, then this provides support for the hypothesis that macroevolution is mainly an accumulation of micro-evolutionary change [3]. Alternatively, if patterns of divergence among populations within a species (i.e., nascent species) are in different directions and of differing magnitude among more divergent species, then this would suggest that macroevolution is somehow distinct from microevolution [2].

Livebearing fishes of the family Poeciliidae have been the subject of numerous studies evaluating the impact of different environments on life history strategies (e.g., [7,8,13–18,22–26]). The effect of divergent predation environments has been of particular interest, with among population patterns of divergence in life history being characterized by a relatively large size at maturity, low reproductive allocation, large offspring size, and low number of offspring in predator-free or low mortality sites contrasted with small size at maturity, high reproductive allocation, small offspring size, and high number of offspring in predator or high mortality sites [22–24,27,28]. Although these patterns are well documented, what is not known is how these patterns compare across the species boundary. In other words, do the patterns of variation observed among populations (i.e., micro-evolutionary patterns) in response to predation environment correspond to the pattern of divergence among species (macro-evolutionary patterns) that occur in different predation environments?

The livebearing fish genus *Brachyrhaphis* has emerged in recent years as a model for understanding trait evolution and natural selection at different stages of divergence [29–33]. Within *Brachyrhaphis*, several species contain populations that occur in different predation environments, where they have independently and repeatedly evolved divergent adaptations in traits such as life-history [23,24] and morphology [30]. For example, populations of *B. rhabdophora* (Regan) from predator environments have evolved life-history strategies that include younger age and smaller size at maturity relative to predator-free populations [23,34]. Similar patterns may be present at deeper levels of evolutionary divergence, for example, between sister species *B. terrabensis* (Regan) and *B. roseni* (Bussing) which have existed in contrasting predation environments over longer evolutionary periods [35]. *Brachyrhaphis roseni* is distributed in lowland streams and rivers along the Pacific coast of southwestern Costa Rica and western Panama. Average body size in *B. roseni* is smaller than in *B. rhabdophora*, and it co-occurs with several other fish species including many predatory species. *Brachyrhaphis terrabensis* occurs in small streams and large rivers in and around the Valle de General in south central Costa Rica and at higher elevations in streams and rivers on the Pacific versant of western Panama. Average body size in *B. terrabensis* is larger than in *B. rhabdophora*, and in many locations where it occurs, there are few (if any) other fish species and relatively few large-bodied piscivores [36,37]. These species

that occur in differing predation environments provide a comparison for the differentiation of life history patterns among species, and corresponding development of reproductive isolation [31,38]. This unique system thus provides an excellent model to test for correspondence between micro- and macro-evolutionary patterns of divergence, where similar agents of selection appear to be driving repeated trait divergence [31], and will help test how generalizable these patterns are when coupled with similar work in closely related species that occur in divergent selective regimes (e.g., [7,8]).

We use these three species to compare patterns of life history divergence in contrasting environments before and after speciation. By comparing the effect of predation environment on life history evolution within a species that inhabits both predator and predator-free environments (*B. rhabdophora*), to two fully-derived species that exclusively inhabit either predator or predator-free environments (*B. roseni* and *B. terrabensis*, respectively), we can compare the effects of predation environment across the speciation boundary. We test the hypothesis that divergent patterns of variation in life history traits observed between predator and predator-free environments within a species (microevolution) should be amplified in species that have fully differentiated into separate species in predator and predator-free environments (macroevolution). In brief, we test the prediction that there would be no difference between micro- and macro-evolutionary patterns in the divergence of life history traits.

2. Materials and Methods

2.1. Collection and Trait Measurement

To compare variation in life history within and among species, we collected samples from multiple locations for each of the three species of *Brachyrhaphis* throughout their range in Costa Rica and Panama. We used data from 22 populations of *B. rhabdophora* (11 from predator-free locations, female N = 263, male N = 474; and 11 from predator locations, female N = 271, male N = 508; data from some of these populations were published in Johnson and Belk [23]), 13 populations of *B. roseni* (female N = 530, male N = 580), and 13 populations of *B. terrabensis* (female N = 287, male N = 545; Figure 1). All samples were collected during the dry season (late April to June). Samples of *B. rhabdophora* were collected in 1996, 1997, and 1998. Samples of *B. terrabensis* were collected in 1997, 2007, 2011, and 2013. Samples of *B. roseni* were collected in 1997, 2005, 2007, and 2011. Within species, all samples were collected from locations that were separated in different streams and drainages, and previous work suggests that each collection represents an independent sample at the population level [38]. We treat location as a random effect in the analysis to adjust for any lack of independence among locations, including years of collection or environmental variation specific to a given location. Previous work indicated that mean life history traits varied little among years, but one trait, namely reproductive allocation, did vary between wet and dry seasons [23]. Restricting our comparisons to samples collected during the dry season avoids potential confounding of seasonal and among location variation in reproductive allocation. We do not have representative samples for wet season analysis. However, based on comparisons made by Johnson and Belk [23], an analysis based on wet season samples would be unlikely to change our general results. Fish were collected with a handheld seine (2 mm mesh), and were immediately preserved in ethyl alcohol.

We designated four species by predation environment population groups for subsequent comparison as follows. *Brachyrhaphis terrabensis* represents the fully-derived species found mainly in predator-free environments. *Brachyrhaphis roseni* represents the fully-derived species found in predator environments. *Brachyrhaphis rhabdophora* populations are divided into two categories representing nascent species in predator-free environments or nascent species in predator environments based on collection location. Hereafter, we refer to these four groups as species-predation groups.

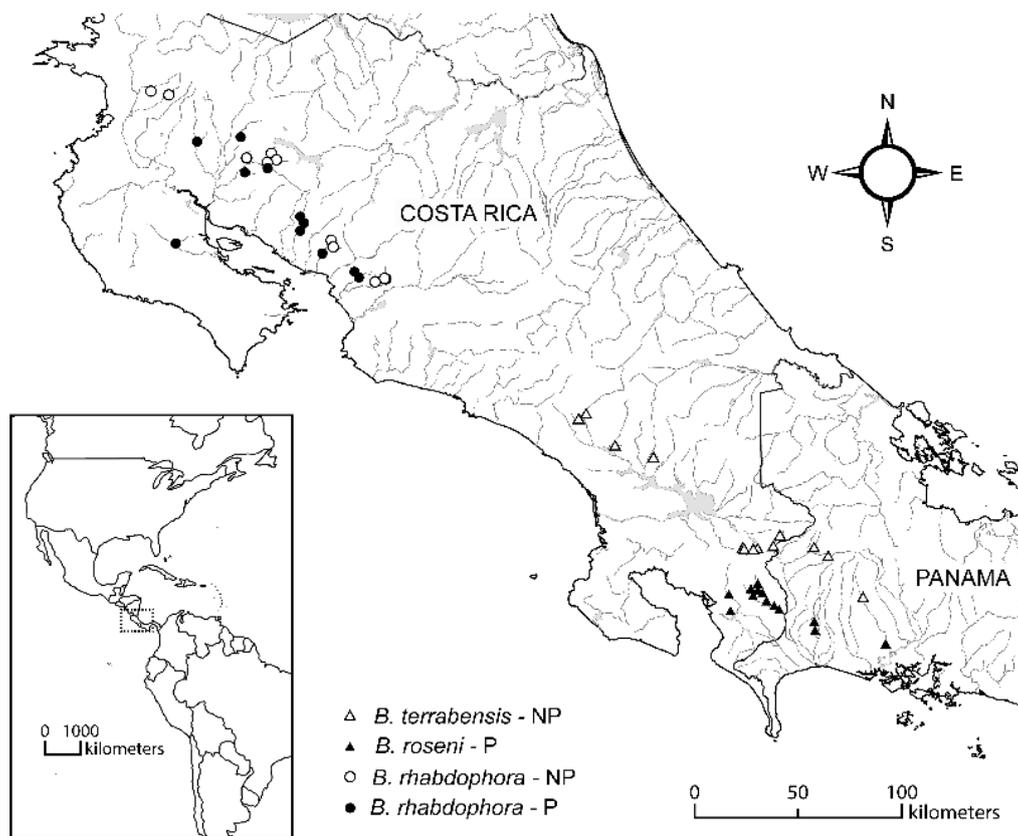


Figure 1. Map of collection locations.

For each sample, we sorted fish into three categories based on characteristics of the anal fin (gonopodium development): mature males (fully developed gonopodium), immature males (developing gonopodium), and females plus juveniles (undifferentiated anal fin). We measured standard length, total length, and wet mass for each individual in the female/juvenile group, and dissected them to directly observe the gonads to determine reproductive maturity. We considered all individuals with mature ova or developing embryos as mature females. For mature females, we removed and counted the number of ova or embryos (clutch size) and assigned developmental stage of embryos according to Haynes [39]. Depending on the number of females available, the entire sample or a subsample of females (we haphazardly selected ~25 females from across the full size range of females available) and their clutches were dried at 50 °C for 48 h and dry mass of the female and her clutch was measured on a digital scale to the nearest 0.1 mg. We removed the entire digestive tract of females before we dried them to avoid bias due to differential fullness of undigested material. Sample sizes among *B. rhabdophora* predator-free locations ranged from 22 to 76 for males and from 15 to 28 for females. Sample sizes among *B. rhabdophora* predator locations ranged from 27 to 63 for males and from 18 to 29 for females. Sample sizes among *B. roseni* locations ranged from 14 to 90 for males and from 8 to 99 for females. Sample sizes among *B. terrabensis* locations ranged from 15 to 89 for males and from 9 to 46 for females.

From the above measurements we derived six life history variables. The variables were: (1) adult male standard length; (2) adult female standard length; (3) minimum reproductive female standard length; (4) reproductive allocation, i.e., dry mass of clutch adjusted by stage of development and female dry mass; (5) mean offspring size, i.e., clutch dry mass divided by offspring number (adjusted by stage of development and female dry mass); and (6) offspring number or clutch size (adjusted for female dry mass).

2.2. Statistical Analysis

To determine how life history traits varied among species-predation groups, we used a mixed model in an analysis of covariance design (mixed ANCOVA). We used individual life history trait values for all individuals from each of the 48 populations as response variables, with the exception of minimum reproductive female size which was analyzed as a population minimum ($n = 48$). All life history trait response variables were \log_{10} transformed prior to analysis to better meet the assumption of normality of residuals. Residual plots were inspected for each model, and residuals met the assumptions of the parametric models. The predictor variables for each of the six life history response variables were (1) predation regime (predator or predator-free environment) and (2) position relative to the speciation boundary or speciation position ('before' for the two types of populations of *B. rhabdophora*, and 'after' for *B. roseni*, and *B. terrabensis*). The interaction between the two main effects was included in the model and used to test for the effect of predation environment on life history over evolutionary time. Reproductive allocation, i.e., clutch dry mass, and offspring size, i.e., mean dry mass of offspring, vary with female dry mass and developmental stage of embryos (all three species are lecithotrophic, so dry mass is expected to decline during development; [40]). To derive a measure of reproductive allocation and offspring size that is independent of developmental stage and size of female across all locations and species, we included the developmental stage of embryos and dry mass of females (\log_{10} transformed prior to analysis) in the model as covariates and included a random effect for their intercept. This adjustment yields least-squares means that are evaluated at a common value for developmental stage and female dry mass across all populations and species. Clutch size varies with female dry mass, so we used the dry mass of females (\log_{10} transformed prior to analysis) as a covariate and included a random effect for the intercept in this model as well. In addition, in all models for all five of the individual-level response variables, we included location as a random effect to account for any degree of non-independence among sampling locations including years of collection or environmental variation specific to a given location. We evaluated differences among species-predation groups by evaluating 95% confidence intervals around the least squares means. If confidence intervals of a given mean did not overlap an adjacent mean the two means were considered significantly different.

If divergence in life history before speciation based on predator environment is amplified after speciation, then this would be indicated by a significant interaction of the two main effects (of course other patterns can generate a significant interaction, but we are focusing on the pattern where life history differences are amplified at later stages of speciation). For example, large relative clutch sizes in the nascent species would correspond to even larger relative clutch sizes in the fully differentiated species in predator environments. The reverse pattern would be observed in predator-free environments, thus smaller relative clutch sizes in the nascent species would correspond to even smaller clutch sizes in the fully differentiated species. The lack of an interaction between the two main effects indicates no differential effect of predator and predator-free environments on life history across the speciation boundary, and consequently, no role of predation environment in life history trait divergence after speciation.

To determine patterns of covariation among life history traits and subsequent patterns among species-predation groups we used principal components analysis (PCA). We used five mean life history trait values for each of the 48 populations as input for the PCA. We excluded the minimum size of mature females as a trait because of the high correlation with mean adult female size. If predation environment accounts for variation in life history among species-predation groups, we would expect the major axis of phenotypic variation in life history, i.e., the first principal component, to correspond to a predation by speciation position gradient. *Brachyrhaphis roseni* and *B. terrabensis* should occupy the extremes of the axis and *B. rhabdophora* populations should occupy the central region of the axis. Furthermore, *B. rhabdophora* populations from predator environments should be closer to *B. roseni* and *B. rhabdophora* from predator-free environments should be closer to *B. terrabensis*. We used SAS version 9.4 (Statistical Analysis Systems, SAS Institute, Cary, North Carolina, USA) to run all statistical analyses.

3. Results

Male and female body size and minimum size of reproductive females varied significantly by predation regime, but not by speciation position, i.e., before and after speciation. The interaction between predation regime and speciation position was significant for all three response variables (Table 1), indicating continued differentiation among predator and predator-free environments before and after speciation. For male and female body size analyses, all means differed significantly from all others based on inspection of confidence intervals. For minimum female body size, *B. roseni* did not differ from *B. rhabdophora* in predator environments, but the other two species-predation groups differed from these two and from each other. In both sexes, predator-free *B. rhabdophora* were larger than predator *B. rhabdophora*, whereas *B. roseni* (predator environment, fully-derived species) and *B. terrabensis* (predator-free environment, fully-derived species) were the smallest and largest of the four species-predation groups, respectively (Figure 2).

Table 1. Analysis of variance table for male size (SL), female size (SL), and minimum size of reproductive females (SL) in response to predation environment and speciation position. Significant values are bolded.

Response	Effect	df num/den	F	p-Value
Male size	Predation environment	1/44.1	68.12	<0.0001
	Speciation position	1/44.1	2.70	0.11
	Predation environment*speciation position	1/44.1	15.88	0.0002
Female size	Predation environment	1/44	87.55	<0.0001
	Speciation position	1/44	1.02	0.32
	Predation environment*speciation position	1/44	22.72	<0.0001
Min. female size	Predation environment	1/44	40.7	<0.0001
	Speciation position	1/44	3.63	0.063
	Predation environment*speciation position	1/44	6.41	0.015

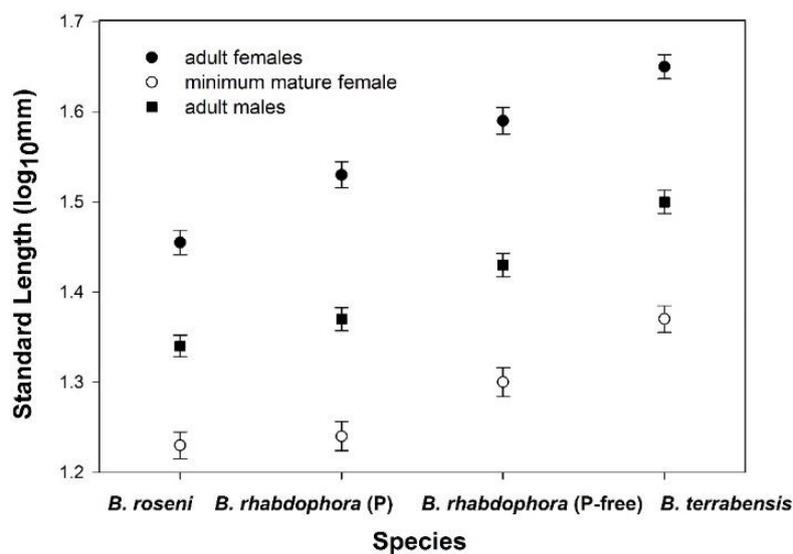


Figure 2. Mean length (\pm 1SE) by species-predation group. Length in mm (\log_{10} transformed) for adult females, adult males, and minimum size of reproductive females.

Similar to body size traits, clutch size and offspring size varied significantly by predation regime, but not by speciation position, i.e., before and after speciation. The interaction between predation regime and speciation position was significant for both clutch size and offspring size (Table 2), indicating

a continuing pattern of divergence among predator and predator-free environments, both before and after speciation. Clutch size was larger in the nascent predator group (*B. rhabdophora*) compared to the nascent predator-free group (*B. rhabdophora*), and clutch size was largest in the fully-derived species in the predator environment (*B. roseni*), and smallest in the fully derived species in the predator-free environment (*B. terrabensis*). Clutch size in the predator-free *B. rhabdophora* locations did not differ from clutch size in *B. terrabensis*, but all other means differed from each other (Figure 3A). Offspring size varied inversely to clutch size, and all means differed significantly from all others. Offspring size was smaller in the nascent, predator group (*B. rhabdophora*) compared to the nascent, predator-free group (*B. rhabdophora*), and was smallest in the fully-derived species in the predator environment (*B. roseni*), and largest in the fully derived species in the predator-free environment (*B. terrabensis*; Figure 3B).

Table 2. Analysis of covariance table for clutch size, offspring size, and reproductive allocation in response to predation environment and speciation position. Significant values are bolded.

Response	Effect	df num/den	F	p-Value
Clutch size	Predation environment	1/37.3	34.6	<0.0001
	Speciation position	1/35.5	1.96	0.17
	Predation environment*speciation position	1/35.8	7.52	0.0095
	Female mass	1/447	869.4	<0.0001
Offspring size	Predation environment	1/44.2	65.02	<0.0001
	Speciation position	1/38.2	0.56	0.46
	Predation environment*speciation position	1/39.4	25.13	<0.0001
	Female mass	1/898	111	<0.0001
	Developmental stage	1/81.5	51.69	<0.0001
Reproductive allocation	Predation environment	1/43.2	4.99	0.03
	Speciation position	1/39.7	0.62	0.44
	Predation environment*speciation position	1/40.3	1.00	0.32
	Female mass	1/637	1084	<0.0001
	Developmental stage	1/56	51.97	<0.0001

Reproductive allocation varied significantly by predation regime, but not by speciation position and the interaction was not significant (Table 2). Reproductive allocation exhibited high variance within species-predation groups compared to other life history traits. Reproductive allocation in *B. roseni* (the fully-derived species in the predator environment) was significantly higher than reproductive allocation in the nascent, predator-free group (*B. rhabdophora*) and in the fully-derived species in the predator-free environment (*B. terrabensis*; Figure 3C).

The first two principal components accounted for 92% of the variation in life history among all 48 population samples (PC1 = 72% and PC2 = 20%). The first principal component exhibited eigenvector loadings of about equal magnitude on four of the five variables: male size at maturity, female size, offspring size, and clutch size, whereas the loading for reproductive allocation is somewhat lower (Table 3). The first principal component represents the predicted pattern of variation in response to a predation gradient—large body size and offspring size, and lower clutch size and reproductive allocation in predator-free environments compared to small body size and offspring size, but higher clutch size and reproductive allocation in predator environments. The four species-predation groups show clear patterns of divergence along the PC1 axis. The two nascent species-predation groups represented by *B. rhabdophora* in predator and predator-free environments are located in the center of the axis with predator populations shifted to the left and predator-free populations shifted toward the right. The two fully-derived species-predation groups occupy the extremes of the axis with *B. roseni* (predator environments) on the left and *B. terrabensis* (predator-free environments) on the right (Figure 4). The second principal component loads heavily only on reproductive allocation, and

thus represents variation in reproductive allocation only (Table 3). On PC2 species show almost complete overlap (Figure 4). Variation among populations on PC2 represents variation within each of the species-predation groups rather than among species-predation groups as observed on PC1.

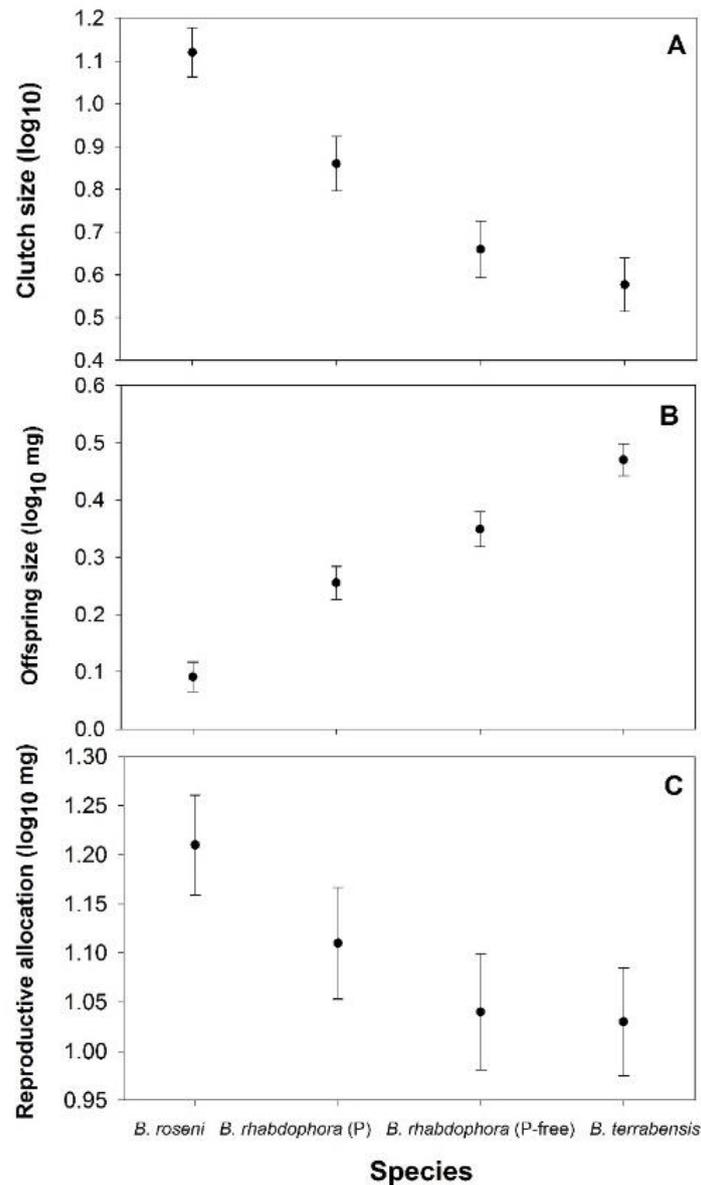


Figure 3. Mean (\pm 1SE) of \log_{10} transformed (A) clutch size; (B) offspring size; and (C) reproductive allocation by species-predation group.

Table 3. Loadings of original life history variables on principal components one and two. Highly influential loadings are bolded.

Life History Variable	Eigenvectors	
	PC1	PC2
Reproductive allocation	−0.33	0.78
Offspring size	0.47	0.26
Clutch size	−0.49	0.34
Male size	0.45	0.38
Female size	0.48	0.26

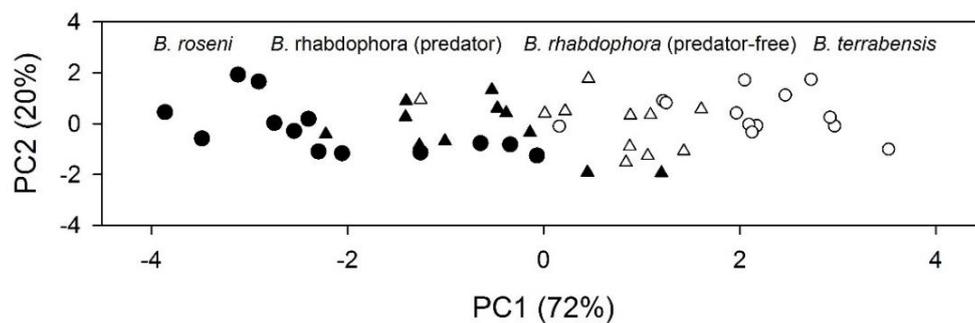


Figure 4. Means on principal components one and two for each sampling location. *Brachyrhaphis roseni* is represented by closed circles, *B. rhabdophora* in predator environments is represented by closed triangles, *B. rhabdophora* in predator-free environments is represented by open triangles, and *B. terrabensis* is represented by open circles. Plot is scaled by the amount of variation explained by each principal component.

4. Discussion

One way to frame the macroevolution–microevolution debate is to suggest that selection within species acts on individuals to create divergence in trait values among populations; whereas, selection among species or higher taxonomic groups, acts on emergent properties of species that cannot be reduced to the individual or population level, e.g., species range size or relative abundance, [2,3]. If macroevolution is thus qualitatively different from an accumulation of micro-evolutionary responses, then life history traits among species of *Brachyrhaphis* may not show patterns consistent with those predicted from a predator/predator-free comparison. Our data suggest that intraspecific trait differences do in fact scale up to the between-species level. In other words, our findings are consistent with the idea that micro-evolutionary diversification mirrors macro-evolutionary diversification. Other researchers have suggested that both micro- and macro-evolutionary mechanisms can operate among taxa and that the question is which of those processes seems to have a greater effect or occur more frequently [3]. Our data support the position that micro-evolutionary processes occurring over sufficient time can explain patterns of variation among species and that it may not be necessary to invoke macro-evolutionary processes, i.e., higher level selection. These results seem to corroborate recent work in poeciliids that has demonstrated a strong correlation between the strength of divergent natural selection, and the magnitude of population differentiation achieved [8].

We found that variation observed among nascent species differentiated by predation environment is a good predictor of variation among established species differentiated by predation environment. This result suggests a central role for selection arising from the predation environment in the divergence of these species over evolutionary time. Patterns observed among populations within *Brachyrhaphis* species appear to be magnified through evolutionary time. Such a pattern could result from the sustained directional selection on life history traits, like that observed over multiple generations as documented in Trinidadian guppies, *Poecilia reticulata* [41], or that observed in species of *Gambusia* and *Poecilia* from habitats with different concentrations of hydrogen sulfide [8]. However, in Trinidadian guppies and in *B. rhabdophora*, the effects of habitat-specific directional selection may be opposed and reduced by homogenizing effects of gene flow. Gene flow, albeit modest, certainly exists between populations in predator and predator-free environments in these systems [42,43]. Over evolutionary time we would expect isolating mechanisms to arise between predator and predator-free environments, which could further diminish gene flow and allow for greater divergence among populations [31], as has been observed in other species within Poeciliidae [8].

What mechanisms might lead to a reduction in gene flow and allow the evolution of isolating mechanisms such that speciation can proceed? Recent work on morphology and performance of these same species of *Brachyrhaphis* shows how predation environment creates reproductive isolation through immigrant inviability. An assessment of alternative morphology–performance–fitness pathways

indicates that selection from predation plays an important role in driving population differentiation and reproductive isolation at early and late stages of divergence [31]. In addition, predation may act directly to reduce gene flow by reducing movement in predator environments [44]. Furthermore, reproductive isolating mechanisms may arise directly from results of selection on life history traits. For example, differences in size at maturity evolve rapidly between predator and predator-free environments due to size specific mortality rates [29,32,41]. Size is also a strong component of female mate choice in many poeciliids [45]. Thus, small males from predator environments may experience reduced mating success in predator-free environments, where the larger predator-free males have an advantage, potentially resulting in reproductive isolation through female discrimination against immigrant males. Reproductive isolation may evolve indirectly as a result of selection on other traits, or tradeoffs with other traits. For example, selection by predators affects patterns of coloration in male Trinidadian guppies leading to reduced levels of coloration in predator environments [46]. In predator-free environments, and to a lesser extent in predator environments, females prefer colorful males. Thus, males from predator environments could suffer reduced mating opportunities in predator-free environments because of their relatively dull coloration [46,47]. Furthermore, body shape could play a role in driving divergence and reproductive isolation between predator and predator-free environments. *Brachyrhaphis* that occur with predators tend to have a more streamlined body with a robust tail region relative to those that occur in predator-free environments [30]. Difference in body shape results in strong differences in swimming ability, with fish from predator environments having much higher burst speed swimming ability, but reduced endurance, relative to predator-free populations [48]. These differences in body shape, and the resulting effects on burst speed, have a direct relationship with survival in the presence of predators, with faster, more streamlined fish enjoying significantly higher survival rates when encountering piscine predators [48]. Given that these patterns have been found in numerous species of poeciliids [49,50], and that female choice can be largely based on differences in body shape independent of body size [51,52], it is reasonable to speculate that adaptive differences in body shape could also influence premating reproductive isolation in *Brachyrhaphis*. Premating reproductive isolation can develop relatively quickly in sympatric settings in African cichlids [53]. Similarly, traits related to or covarying with life history may result in rapid reproductive isolation in poeciliids in contrasting environments.

Theoretically, reproductive allocation is predicted to vary between predator and predator-free environments [54–56]. High reproductive allocation is predicted to be favored in predation environments, while low reproductive allocation is predicted to be favored in predator-free environments. This prediction is met in Trinidadian guppies, which exhibit clear and repeated patterns of differences in reproductive allocation between predator and predator-free environments [22]. However, populations of *B. rhabdophora* found in predator and predator-free environments do not show differences in reproductive allocation ([23], this study). In contrast, the fully-derived species (*B. roseni* and *B. terrabensis*) show a significant difference in reproductive allocation and the pattern is consistent with expectations from theory [54–56]. Reproductive allocation is highly variable among locations within species-predation groups in this study, suggesting that some other factor, in addition to predation environment, influences reproductive allocation.

Two other selective influences may constrain the evolution of reproductive allocation in response to predation environment in these species. First, reproductive allocation varies among populations in these species-predation groups apparently in response to resource availability [23], a pattern observed in several species of poeciliids [25,57]. Resources in streams and rivers of Central America can vary dramatically between wet and dry seasons [58,59], creating an annually fluctuating pattern of resource availability. Temporal variability in the environment may select for plasticity in response to resource environment [60], and this effect may overwhelm selective effects on reproductive allocation in response to predation environment. Second, reproductive allocation may vary as a function of ontogeny over the reproductive lifespan. Predictions from the cost of reproduction hypothesis [61] suggest that young females will allocate relatively less to current reproduction compared to future reproduction; whereas

older females will allocate more to current reproduction because their future opportunities are limited. This within-lifetime variation is obscured by the use of population means combining females from many different age classes. Different types of predation environments may select for different patterns of allocation over a lifetime. In *B. rhabdophora*, females in predation environments exhibit little change in reproductive allocation over their lifetime. In contrast, females in predator-free environments exhibit patterns consistent with the cost of reproduction hypothesis, i.e., young females show low levels of reproductive allocation (less than young females in predator environments) and old females show high levels of reproductive allocation (more than old females in predator environments [62]. Predation environment may select for the pattern of allocation over a lifetime rather than for differences in the mean overall. Thus, mean reproductive allocation may operate somewhat independent of predation environment because of the influence of temporal variability on resource environment and variation in pattern of reproductive allocation within an individual's lifetime.

In summary, patterns of life history divergence in response to predation environment within and among closely related species of *Brachyrhaphis* fishes support the idea that an accumulation of micro-evolutionary changes are sufficient to explain divergence both before and after speciation in this group. Predation environment selects for differences in life history that are magnified through evolutionary time on both sides of the speciation boundary. There is no evidence for selection at higher taxonomic levels or on emergent properties of species, i.e., distinct macro-evolutionary processes. Given the large number of poeciliid species and their distribution across a wide range of contrasting environments, further comparisons similar to our study and those conducted recently in poeciliids from other types of divergent environments [7,8] may be possible among species and populations of poeciliids, providing a test of generality for our results [63].

Author Contributions: Conceptualization, M.C.B., J.B.J.; Data curation, M.C.B., S.J.I.; Formal analysis, M.C.B.; Funding acquisition, M.C.B., S.J.I., J.B.J.; Investigation, M.C.B., S.J.I., J.B.J.; Methodology, M.C.B., S.J.I., J.B.J.; Project administration, M.C.B., J.B.J.; Resources, J.B.J.; Supervision, M.C.B.; Visualization, M.C.B., S.J.I.; Writing—original draft, M.C.B., S.J.I., J.B.J.; Writing—review & editing, M.C.B., S.J.I., J.B.J. All authors have read and agreed to the published version of the manuscript.

Funding: Several Mentoring Environment Grants, a Sant Endowment Grant, and the Mollie and Karl Butler Young Scholar Award, all from BYU, helped support field work. SJI was supported by a National Science Foundation Graduate Research Fellowship while at BYU. Additional funding came from a National Geographic Young Explorers Grant, a Theodore Roosevelt Memorial Grant from the American Museum of Natural History, an Exploration Fund Grant from The Explorers Club, and a student research award from the American Society of Naturalists, all awarded to SJI during graduate school.

Acknowledgments: Data for this project have been compiled over the past three decades. Over this time, we are especially grateful for the cooperation of Javier Guevara Sequerra at the Vida Silvestre de Costa Rica who issued collecting permits, and to Bill Bussing for helpful insight on Costa Rican fishes as we started this work. We thank the BYU Biology Department and College of Life Sciences for support to involve undergraduates and graduate students in this long-term research project. Thanks to numerous students who helped with field collection and laboratory dissection of samples. All collections of fishes for data contained in this paper were done according to Guidelines for The Use of Fishes in Research provided by The American Fisheries Society, the American Institute of Fisheries Research Biologists, and the American Society of Ichthyologists and Herpetologists. Earlier collections referenced in this paper were done prior to IACUC required protocols for field research. Later collections were covered by BYU IACUC protocols 11-0901 and 12-0607.

Conflicts of Interest: The authors declare no conflict of interest.

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