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Multispecies Character Displacement in Mexican *Poeciliopsis* Fishes

Andrea J. Roth

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

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ABSTRACT

Multispecies Character Displacement in Mexican *Poeciliopsis* Fishes

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Doctor of Philosophy

Competition has long been recognized as a central force in shaping evolution, particularly through character displacement. Yet research on character displacement is biased as it has focused almost exclusively on pairs of interacting species while ignoring multispecies interactions. Unfortunately, communities are seldom so simple that only pairs of species interact, and it is not clear if inferences from pairwise interactions are sufficient to explain patterns in nature. A more realistic approach is to ask how traits evolve when multiple species interact. Here I explore the importance of multispecies competitive interactions on trait evolution in four congeneric species of livebearing fishes in the genus *Poeciliopsis* (*P. prolifica, P. viriosa, P. latidens,* and *P. presidionis*). These species are found co-occurring throughout northwestern Mexico:. My first chapter builds a framework for multispecies character displacement research by hypothesizing three effects that an unconsidered competitor, termed a hidden competitor, can have on pairwise interactions and the resulting pattern of character displacement. I show through these effects that research focused solely on pairwise interactions can be misleading for character displacement. I also provide suggestions on how to address character displacement research that incorporates more complexity. In chapter two, I test for character displacement in body shape in the four congeneric species. I found evidence for convergent character displacement in populations of *P. prolifica*, *P. viriosa,* and *P. latidens*. I also found that the convergence in body shape was not consistently in the same direction, meaning that when more than two species co-occurred I did not find a more extreme body shape that when only two species co-occurred. On the contrary, body shape when more than two competitors co-occurred seemed to be intermediate between the shape of two competitors and no competitor. This intermediate shape suggests that evolution in multispecies communities may occur in response to several competitors, rather than pairwise interactions. Finally, in chapter three, I test the effect of several hypothetical selective pressures on life history of *P. prolifica*, including intraspecies and interspecies competition, factors not often considered in life history evolution. I found that competition, both intraspecific and interspecific, was the most important factor in explaining variation in life history. I also found that the best models were those that included these selective pressures as direct effects as opposed to indirect effects through resource availability. However, it is not clear why competition was supported as a direct effect and future studies are needed to fully understand this aspect. Overall, my research suggests that competition plays an important role in shaping trait evolution, even in traits where it has not been considered. Thus, competition should be included in future studies as it may be an important factor in shaping several traits. I also found that competition in multispecies interactions is more complex than in a simple pairwise interactions, and can be harder to detect due to confounding effects acting in conjunction with competition. My study highlights the importance of competition and of considering multispecies competition to better understand the effects of competition.

Keywords: character displacement, competition, livebearing, *Poeciliopsis*, evolution

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Chapter 1

Multispecies character displacement: Approaching reality in competition studies

Andrea J. Roth-Monzón[1*] and Jerald B. Johnson[1,2.]

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Abstract

Competition has long been recognized as a central force in shaping evolution, both through competitive exclusion and character displacement. Although initially contested, character displacement has now gained widespread support and broad interest from the scientific community. However, research on character displacement suffers from bias in that it has focused almost exclusively on pairs of interacting species while ignoring multispecies interactions (more than two). Communities are seldom so simple that only pairs of species interact and even when interactions are primarily pairwise the co-occurrence of other species can have indirect effects. Thus, a more realistic approach is to ask how traits evolve when multiple species interact. Here we hypothesize three effects that an unconsidered competitor—which we term a 'hidden competitor'—can have on pairwise interactions and the resulting pattern of character displacement. We show through these effects that research focused only on pairwise interactions can be misleading for character displacement, due to the unconsidered effects of hidden competitors. We also provide suggestion on how to tackle character displacement research that incorporates more complexity. We anticipate that focusing on hidden competitors will motivate researchers to consider additional complexity in the study of character displacement, thereby better understanding trait evolution in natural systems.

Introduction

Competition is an important ecological driver that can shape communities in nature, either by competitive exclusion or by causing character displacement that allows species to co-exist (Pfennig and Pfennig 2010). Thus, this process has captured the interest of both evolutionary and community ecologists for several decades (Pfennig and Pfennig 2010; Stuart and Losos 2013). Character displacement occurs when traits displace (diverge or converge) in a way that reduces negative competitive interactions (Brown and Wilson 1956; Grant 1972, Pfennig and Pfennig 2012). Although character displacement is divided into different types, depending on the type of competition considered (e.g. reproductive character displacement, ecological character displacement, and agonistic character displacement; Pfennig and Pfennig 2012), here we focus on a general definition of character displacement that encompasses each of these forms.

Character displacement has been the focus of hundreds of studies (Schluter 2000a; Schluter 2000b). Although this phenomenon was initially contested, it now enjoys widespread support and broad interest within the scientific community (Stuart and Losos 2013). This is demonstrated by the steady increase in publications on this topic over the past three decades (Fig. 1.1). This said, research on character displacement has suffered from a known bias wherein it has focused almost exclusively on pairs of interacting species, avoiding more complex interactions among three or more competing species (Fig. 1.1). This trend is not surprising in that early tests of character displacement started with the simplest competitive interactions between two species, simply to see if the process occurred. However, the reality is that in many natural systems, competitive interactions occur among multiple (more than two) species simultaneously. In fact, even when the dominant interaction in a community is between two

species, the presence of other species can cause indirect effects that alter these pairwise interactions (terHorst et al. 2018). Moreover, recent research suggests that trait displacement can differ when multiple species interactions are considered relative to focusing on pairwise interactions alone (Levine et al. 2017; terHorst et al. 2018). Hence, we have reached a point in this field where it is helpful to consider how traits displace when more than two species interact.

Here, we examine character displacement in a multi-species context. We do this by developing a framework wherein we evaluate the effects that an unconsidered competitor (which we call a 'hidden competitor') can have on competitive pairwise interactions and the resulting nature of trait displacement in the pairwise species. We show that a hidden competitor can cause unexpected trait displacement patterns, including asymmetrical divergence, and even character convergence—patterns that are sometimes inexplicably found in pairwise interactions (Cooley 2007; Jang 2008). We also show how a hidden competitor can lead to an unusual trait displacement pattern that we term cascade divergence. Given these findings, we argue that a more realistic approach to studying character displacement should consider all potential competitors in natural systems, even when some of these competitive interactions might be weak relative to others.

Effects of hidden competitor

Hidden competitors can have at least three effects on pairwise competitive interactions: (1) niche crowding; (2) non-hierarchical competition; and (3) cascade effects. We focus on these three effects because they are unique to multispecies interactions. In order to understand how a hidden competitor can cause different trait displacement patterns, we first consider how it can

exert a negative effect (or cost) in a pairwise interaction, and how trait displacement occurs to mitigate this negative interaction. For each effect, we explain the hypothetical effect and what characteristics change the strength of its negative effect. We also consider the costs associated with each effect and the trait displacement patterns that can result in the species involved in the pairwise interaction.

Niche crowding

Niche crowding occurs when one or more hidden competitors impact the availability of niche space for the species involved in pairwise competition (Simpson 1953; Schluter 2000a, terHorst et al. 2010; Losos 2010). We here consider the niche to be an n-dimensional hypervolume as describe by Hutchinson (1957). We also consider that phenotypic traits can be used as a surrogate to describe how the niche is utilized, as it is done in most character displacement studies (Pfennig and Pfennig 2010). Hence, patterns of trait displacement reflect the effects of competition for the niche. The strength of niche crowding depends on a hidden competitor's niche position and the number of co-occurring hidden competitors (Scheffer and van Nes 2006; Fox and Vasseur 2008). High niche crowding occurs when the hidden competitor's niche is close to the niches of the pairwise competing species. Niche crowding also increases as the number of hidden competitors increases (Fig. 1.2A), as this will limit the amount of available niche space.

The main effect of niche crowding by a hidden competitor is to reduce the niche space available for competing species to diverge (Green 1971; May 1974). It is important to note that a hidden competitor may only crowd one dimension of the niche (Hutchinson 1957), in which case

the species can avoid competition by diverging in the trait that will change its niche use in any niche dimension that is not crowded. However, if the hidden competitor is causing niche crowding then trait displacement can be either divergent or convergent depending on the degree of crowding by the hidden competitor and how this reduces niche availability. If niche crowding is such that the niche is fully occupied, trait displacement in the pairwise species can actually be convergent (Fig. 1.2A), since divergence could lead to greater niche overlap with a hidden species that functions as a stronger competitor than the pairwise competitors (terHorst et al. 2018). High niche crowding can also lead to a niche contraction (Fig. 1.2A) so that the niche of the pairwise species is narrower to avoid overlap with hidden competitors (Colwell 1975; Craig MacLean et al. 2004). In this case, instead of convergence we would see a reduction in trait variance in the pairwise species, likely leading to a more specialized species. Thus, when there is high niche crowding there are two possible responses: trait convergence or trait reduction. In contrast, if crowding is lower so that there is open niche space available then divergence can still occur without overlapping the hidden competitor (Fig. 1.2B). When there is open niche space available due to lower niche crowding, the addition of hidden competitors may actually increase competition and as a result, increase the degree of trait divergence. When this occurs, a higher degree of divergence is expected, which is amplified when the number of hidden competitors increases (MacArthur and Levins 1967; Case 1981).

Niche crowding can also lead to character displacement asymmetries depending on the number and position of the hidden competitors. Asymmetries are common in pairwise character displacement and they are defined by trait displacement occurring in only one of the species being considered, or greater trait displacement in one of the species being considered relative to the other (Schluter 2000a; Cooley 2007). In pairwise studies, asymmetries are considered to be

mostly due to differences in competitive abilities (Schluter 2000a; Cooley 2007). However, asymmetries in character displacement can also be due to a hidden competitor affecting the potential niche of only one of the species in pairwise competition (Fig. 1.2A), and not only different competitive abilities. In order to investigate potential asymmetries, niche position of the hidden competitors need to be considered as it could affect only one species and cause asymmetries, or both species. If both species are affected equally by the hidden competitor asymmetries are not expected to occur. Again, it is also possible, that crowding constrains only one dimension of the niche, so that divergence does not occur in other dimension of the niche and as such the traits linked to that niche use will not diverge. This could lead to asymmetrical trait displacement. However, if the niche is multidimensional, when only one dimension is affected, it is possible that no displacement (convergence or divergence) is found in that trait because trait differences alter niche use in other dimensions of the niche and are sufficient to reduce negative competitive interactions for the species. However, if other trait differences are not sufficient to avoid competition, displacement may still occur. Thus, asymmetries can occur in a single trait or several traits of the species when a hidden competitor is considered.

Non-hierarchical competition

Non-hierarchical competition occurs when there is no single overall winner in multispecies competitive interaction, resulting in competitive interactions that are circular (species A is better than B, B is better than C, and C is better than A) and not linear (A is better than B, B is better than C). Non-hierarchical competition can differ depending on the number of competitors, degree of competition and number of interactions or loops (see Gallien et al. 2007

for a more in deep explanation of non-hierarchical loops). That said, there are two main characteristics of non-hierarchical competition that influence how traits displace in multispecies interactions: (1) the degree of competition; and (2) the presence of nested loops (i.e. one loop inside of another loop; Fig. 1.3A). Depending on such characteristics, non-hierarchical competition can result in no trait displacement, convergence, or asymmetries.

When the degree of competition is equal among competing species (A>B>C>A; 1.3A) in a non-hierarchical loop, trait displacement will not occur because every competitor is affected equally by competition. That is, there is no overall dominant species so each can co-exist without the need for further divergence (Levine et al. 2017). However, if there is any selective pressure to increase competitive ability in one of the species relative to the others, then convergence can occur for all species. This is because convergence usually results in increased competitive ability. Therefore any change in one competitor will destabilize the system, requiring all competitors to improve their competitive ability to co-exist, so if one competitor traits converge so will the other competitors traits to maintain the same relationship and equilibrium. Under this scenario, the competitive network enters an arms race and will follow Red Queen dynamics (Lankau and Strauss 2007; Klauschies et al. 2016) wherein each species has to change in order to maintain a constant competitive position with other competing species. In contrast, a scenario with unequal competition, in which one or more competitors has a higher degree of competition, will results in trait asymmetries. Unequal competition can be due to one species having greater competitive abilities and thus a greater effect on one of the species (A>B>C>>A; Fig. 1.3B). Given that competitive abilities can differ, it is possible for one of the competitors to have a greater ability than the others. The differential degree of competition can cause a higher cost for at least one of the species (A) than for all other competitors (Gallien et al.

2017). When an unequal degree of competition is present, the species paying the higher cost should experience a higher degree of trait divergence (A; Fig. 1.3B).

The type of loops in a non-hierarchical competition network can also create trait asymmetries because nested loops (i.e. one loop inside of another loop; Fig. 1.3B) can affect the degree of competition of the other species in the competitive interaction. A nested loop can cause higher competition for one or more of the competitors, because it will cause a species to be affected by more than one hidden competitor as this species is part of two different competitive interactions (Two loops; A>B>C>A and A>B>C>D>E>F>A; Fig. 1.3B). When nested loops occur, they will create asymmetries in which the need to diverge will be higher for the species that is withstanding more negative interactions due to competition (A is affected by both C and F; Fig. 1.3B). Furthermore, asymmetries due to nested loops can change interactions in the main competitive loop, because when one of the species diverges due to the added degree of competition, this will change its position in the loop and its interaction with the other species. However, how each species changes and its effect on the competitive network will be specific to the system under study.

Cascade effects

A cascade effect is an inevitable chain of effects due to an action affecting the competitive network. Cascade effects in character displacement occur when the effect of character displacement leads to increased competition with other co-occurring species. The cascading effect is triggered when trait divergence within a pairwise interaction causes one of the species to come into competition with a hidden competitor. This hidden competitor will in turn

diverge, and come into competition with a separate hidden competitor, thus, creating a cascade of divergent character displacement (Fig. 1.4). A cascade effect will always result in divergent character displacement because it causes trait divergence that will shift a species niche so that it affects other co-occurring species. In a cascade effect, the hidden species need not be in competition from the beginning. As such, our initial competitive network might include only two interacting species, but the final network could include several others. However, for a cascade effect to occur, niche crowding must be high but with some available niche space, such that niche space is limited but still allows a competitor to diverge. Only in such conditions can a competitor diverge and potentially overlap with a hidden competitor.

Two main characteristics determine the length and degree of a cascading effect: (1) the amount of niche crowding; and (2) the number of competitors present. Higher niche crowding and a higher number of competitors co-occurring lead to longer and greater cascade effects. Thus, when more species co-occur that are close in niche space, the cascade will be longer. This is because it will be more likely that a species diverges into a space that automatically causes increased competition with another species. It is important to recognize that a cascade might only happen in one dimension of the niche or might not happen at all if only one dimension is affected. This is because there might not be sufficient niche overlap in that dimension to cause the need to diverge to avoid negative effects from competition. Cascading effects in character displacement are probably more common in communities with limited niche space, as any small divergence will cause competition with co-occurring species. The ubiquity of cascading effects is unclear, as usually in character displacement studies little is known about all the co-occurring species.

How to study multispecies character displacement

Recognizing that competitive interactions are rarely limited to just two interacting species has several important implications for how we study character displacement. First, researchers studying character displacement should focus on identifying the niche position of each competing species and non-competing species, to understand the available open niche space. This will facilitate predictions regarding the direction and magnitude of trait displacement patterns caused by direct and indirect competitive interactions. Second, researchers should focus on more than one trait to fully understand character displacement, given that the niche is multidimensional and species may not show displacement in the niche dimension measured by a particular trait. Finally, researchers should work to identify all possible competitors in a community, even those with negligible effects, given the potential effects that hidden competitors can have.

Studying multispecies character displacement creates methodological and analytical challenges. First, considering several traits adds variables to the analysis. Second, calculating niche position for all species in a community is challenging, especially for species rich communities. Finally, identifying natural multispecies systems seems particularly challenging, given that such systems need to have co-occurring species that are similar (there is potential for competition), allopatric locations (to compare effect of competition), and localities with combinations of co-occurring species (to determine the effect of each competitor). Nonetheless, tools are available to address these challenges. Multivariate analyses (Green 1971) can aid in the inclusion of several traits in one analysis to account for the multidimensional niche. Surrogates of niche position could be used such as species relatedness or functional similarity (Violle et al. 2011; Tucker et al. 2018) when the data can not be directly obtained. Moreover, researchers can

find natural systems by taking advantage of the increasing availability of species distributions maps in online platforms, which can provide information on potential communities that will have the aforementioned characteristics. In situations in which allopatric localities are not available, researchers could use ancestral trait inference or experimental evolution to infer the trait in the no competition condition.

Aid can also come from using the wealth of knowledge that community ecologists have generated; their studies can inform us of the degree and type of competition (hierarchical or non-hierarchical) from allopatric and sympatric combinations of competing species (for example; Munday et al. 2001; Palmer 2003). Evolutionary ecologists and community ecologists will greatly benefit from working together to understand both what promotes coexistence and what causes certain types of trait displacement. Although the above suggestions will aid observational studies, which are important as they allow for direct measurement of traits from the field, they are often insufficient—most observational studies focus on a single point in time and it can be challenging to discriminate cause and effect. That said, two available tools could aid in this aspect of evaluating multispecies character displacement: simulation models and experimental evolution.

Both simulation models and experimental evolution allow us to control a community in such way that we can manipulate competition and see its effects while minimizing other confounding variables. Thus, each effect considered here could be tested in such way and the resulting pattern confirmed in several replications. In this way we could understand how competition drives trait divergence and how general such patterns are by simulating different communities. Understanding the generalities of such patterns and its link to the process of competition should aid observational character displacement studies that tend to be pattern-

driven due to their comparative framework. However, not all communities can be addressed in a laboratory setting, and an individual-based simulation in which specific individual differences can be introduce can aid to add realism to systems that are hard to study in a controlled laboratory setting (Peck 2008; Railsback and Wolker 2012).

Conclusions

Current research focused only on pairwise interactions among species can be misleading for character displacement, as hidden competitors can cause both direct and indirect effects in a community. Moving forward will require the integration of more complexity in competition to better approach reality. We have provided a basic framework for researchers to use to consider hidden competitors in character displacement and specific trait displacement patterns. The extent to which empirical data align with these hypotheses awaits empirical research focused on multispecies interactions.

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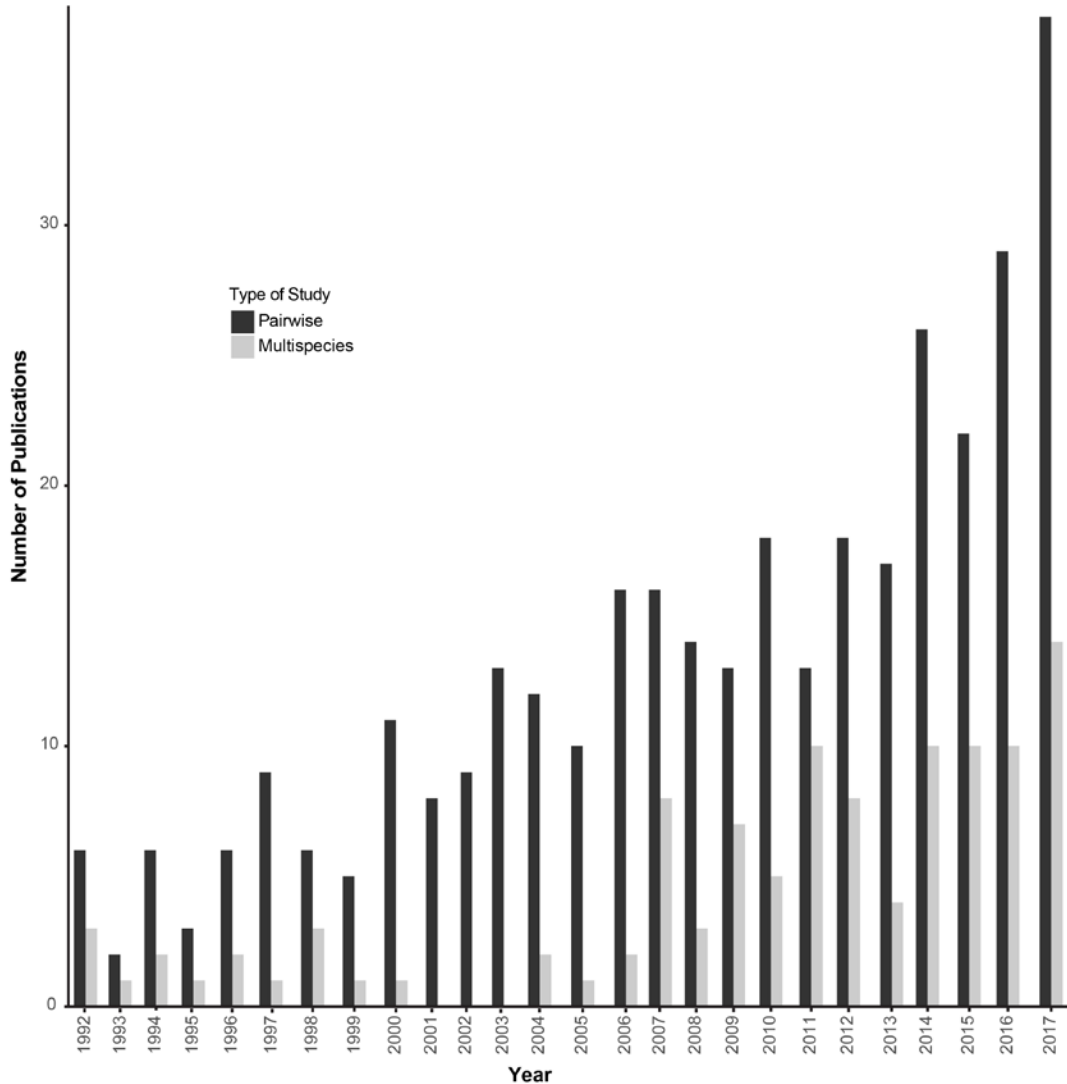
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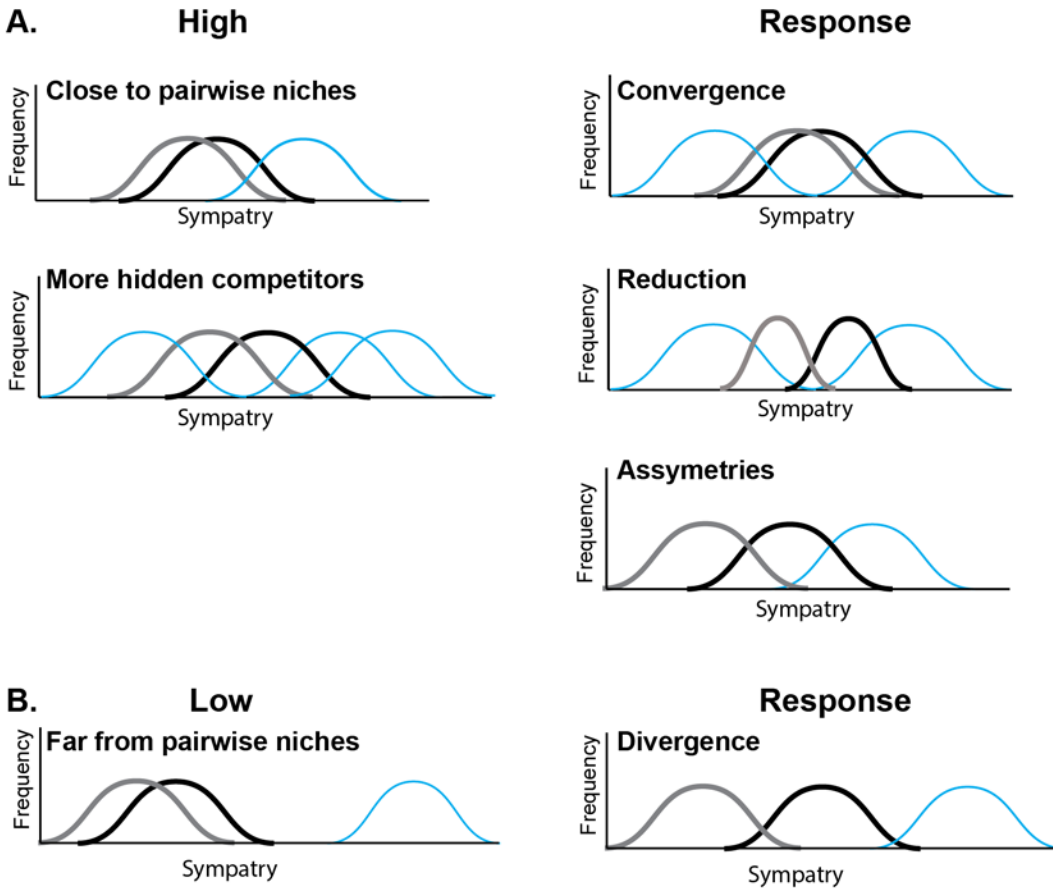
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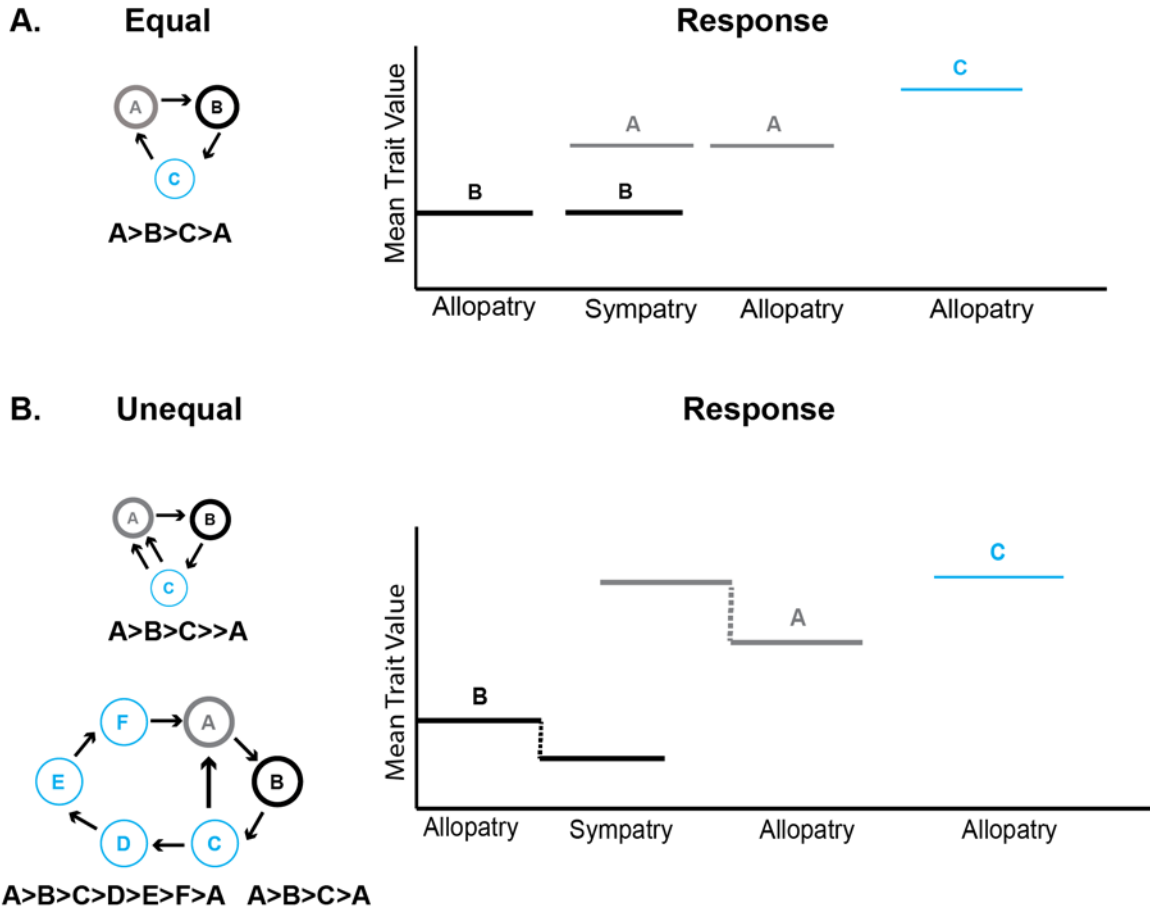
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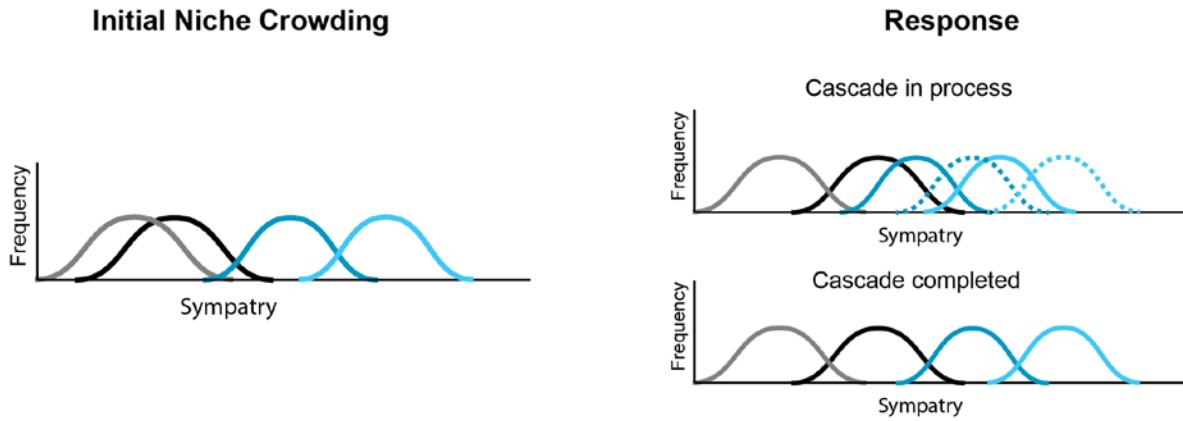
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Chapter 2

Beyond pairwise interactions: Multispecies character displacement in Mexican freshwater fish communities

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Abstract

Competition has long been recognized as a central force in shaping evolution, particularly through character displacement. Yet research on character displacement is biased as it has focused almost exclusively on pairs of interacting species, while ignoring multispecies interactions. Unfortunately, communities are seldom so simple that only pairs of species interact, and it is not clear if inferences from pairwise interactions are sufficient to explain patterns of phenotypes in nature. Here we test for character displacement in a natural system of freshwater fishes in western Mexico that contains up to four congeneric species of the genus *Poeciliopsis.* We focused on body shape. Surprisingly, we found evidence for convergent character displacement in populations of *P. prolifica, P. viriosa* and *P. latidens*. We also found that the convergence in body shape was not consistently in the same direction, meaning that when three or more competitors co-occurred, we did not find more extreme body shapes compared to when there where only two competitors. Rather, when three or more competitors co-occur, body shape was intermediate to the shape found with a pair of species or no competitor present. This intermediate shape suggests that evolution in multispecies communities likely occurs in response to several competitors, rather than to simple pairwise interactions. Overall, our results suggest that competition among multiple species is more complex than a simple set of pairwise competitive interactions. We also explore the challenges of detecting competition in a multispecies framework due in part to confounding environmental effects that act along with competition.

Introduction

Species interactions are known agents of selection that can driver evolution. Several

studies have demonstrated trait evolution caused by species interactions such as predation

(Reznick and Endler 1982; Johnson and Belk 2001; Reznick et al. 2001; Ingley et al. 2014),

parasitism (Buckling and Rainey 2002; Spottiswoode and Stevens 2010), mutualism (Gracia-

Lázaro et al. 2018; Keller and Lau 2018), and competition (Ellis et al. 2015; Roth-Monzón et al.

2017). Competition is also known to drive speciation, in some cases leading to species

radiations, and can be an important factor in the assembly of ecological communities (Pfennig

and Pfennig 2012*a*). It has thus received much attention from both community and evolutionary

ecologists (Pfennig and Pfennig 2010; Stuart and Losos 2013). In ecological communities,

natural selection is thought to act tin a way that reduces competition, thereby promoting species

co-existence (Pfennig and Pfennig 2012*a*).

Trait evolution that reduces competitive interactions is called character displacement

(Brown and Wilson 1956; Grant 1972; Schluter and McPhail 1993; Schluter 2000). Three forms

of character displacement have been described, each defined by a different type of competitive

interaction: ecological character displacement (ECD; Brown and Wilson 1956; Pfennig and

Pfennig 2010); reproductive character displacement (RCD; Butlin 1995; Gröning and Hochkirch

2008); and agonistic character displacement (ACD; Grether et al. 2009; 2017). Here we focus on

ECD, which is caused by indirect competition for resources (food, habitat, etc.) and usually

causes divergence between species to avoid niche overlap (Pfennig and Pfennig 2010).

However, ECD can also cause convergence increasing niche overlap between species (Grant

1972; Pfennig and Pfennig 2012*b*). Most ECD studies focus on pairwise interactions between

species (but see Lemmon and Lemmon 2010; Miller et al. 2014; Grant 2017). Studies of

complex competitive interactions (defined here as competition among three or more species) have received less attention, and it is still unclear how multiple species interactions drive trait evolution (terHorst et al. 2018).

Understanding complex species interactions is important because most ecological communities are composed of multiple interacting species. Moreover, recent evidence suggests that multi-species competition can be fundamentally different from pairwise species competition (terHorst et al. 2018). For example, one species can alter the effect that a second species has on a third species, creating a non-additive interaction, such that trait evolution cannot be inferred by simply knowing the effect of each pairwise interaction. Interestingly, the effects of multispecies competition can remain even when pairwise interactions are the dominant selective force, due to indirect effects of other co-occurring species (terHorst et al. 2018).

How then should character displacement occur where more than two competitors interact? Interestingly, two opposing predictions emerge, each dependent on whether or not the presence of additional competitors cause niche saturation (i.e. when the niche space is filled by species; MacArthur and Levins 1967; Scheffer and van Nes 2006). When the addition of competitors causes niche saturation, then traits should converge or not shift at all. This is because when the niche is saturated by co-occurring competitors, it will constrain trait divergence as the niche space will be unavailable for the focal species to diverge (Scheffer and van Nes 2006; Fox and Vasseur 2008; terHorst et al. 2010). Moreover, this effect should become more pronounced as the number of competitors increases (Hubbell 2006; Scheffer and van Nes 2006). Alternatively, if there is available niche space, then theory predicts that traits will diverge, and divergence will increase with the addition of more competitors (Slatkin 1980; Abrams 1983; terHorst et al. 2018).

Here we examine a natural system of four livebearing fish species from the genus *Poeciliopsis* to determine if and how character displacement occurs in a multi-species context. We evaluate body-shape traits that have long been used in studies of character displacement and are known to be affected by competitive interactions (Schulter and McPhail 1992; Adams and Rohlf 2000; Adams 2004; Husemann et al. 2014). Changes in body shape are expected in response to resource use and habitat use (Rüber and Adams 2001; Aguirre and Bell 2012; Meyers and Belk 2014). We focus on three fundamental questions in this system: (1) do these co-occurring species of *Poeciliopsis* show character displacement; (2) what is the pattern of character displacement among these *Poeciliopsis* species; and (3) how does the number of co-occurring species affect the magnitude or direction of character displacement?

Methods

Study system

Poeciliopsis prolifica, P. viriosa, *P. latidens*, and *P. presidionis* co-occur through western Mexico on the Pacific slope from the Rio Yaqui, Sonora south to near Las Varas, Nayarit (Fig. 2.1; Miller et al. 2005). Although these four species belong to the same genus, they are not sister species. Both *P. prolifica* and *P. viriosa* belong to a strictly northern clade, while *P. presidionis* and *P. latidens* belong to predominantly southern clades. Northward dispersal of the ancestor of *P. presidionis* and *P. latidens* is hypothesized to be relatively recent (2.8 to 6.4 m.y.a.; Mateos et al. 2002). General accounts indicate that these four species are ecologically similar—they all inhabit the mid-water column in streams and small rivers; they are similar in body form; and they are omnivorous, consuming plant and animal matter (Miller et al. 2005). Furthermore, we collected all four species from the same microhabitats while conducting fieldwork. However, to

our knowledge there are no published accounts of the degree of similarity or the competitive

overlap among these four species. Based on their close phylogenetic relationship and ecological

similarity, we conclude that there is potential for competitive interactions. Hence, we use the

number of co-occurring species as an indicator of level of potential competition in our study.

Study sites

We collected females of all species of *Poeciliopsis* with a hand-held seine net (1.3 m x 5

m; 8 mm mesh size) during the dry season: October 2007 and November 2015. Our intent was

to obtain as many independent replicates and combinations of co-occurrence of all four species

as possible. This said, the number of replicates and combinations of species that were available

in the field differed among species (Fig. 2.1, Table 2.1). For each location, we also gathered data

on two potentially influential environmental variables: canopy cover and stream slope (Table

2.2). Canopy cover and stream slope serve as proxies for resource availability and stream

velocity, which can affect body shape (Langerhans and Reznick 2010; Scharnweber et al. 2013).

We estimated canopy cover with the use of a hand-held densitometer at each collection location.

We calculated stream slope for each location in Arcmap (ESRI, Enviromental Systems Research

Institute 2014). We calculated stream slope as the difference between upper elevation and lower

elevation of a 2 km segment of stream (Table 2.2). These locations were similar in terms of

other abiotic properties. For example we found no differences among locations for the different

number of competitors for pH, temperature and conductivity (pH: F= 0.719, P= 0.51;

temperature: F=3.105, P=0.08; conductivity: F=3.278, P=0.09). We also found no potential

piscivorous predators in the locations sampled. However, in all locations (with the exceptions of

site number 3) we found another species of livebearer (*Poecilia butleri*). We also found one location (site number two) with an introduced species of livebearer *Gambusia affinis*.

Quantifying body shape

To quantify body shape, we used a geometric morphometric approach (Rohlf and Marcus 1993). We digitized fourteen biologically homologous landmarks on a lateral image for each fish included in the analysis using the computer software tpsDig2 (Rohlf n.d.). Landmarks were defined as: (1) anterior tip of the snout; (2) anterior extent of the eye; (3) posterior extent of the eye; (4) semi-landmark midway between landmarks 1 and 5; (5) semi-landmark midway between landmarks 4 and 6; (6) anterior insertion of the dorsal fin; (7) dorsal origin of the caudal fin; (8) ventral origin of the caudal fin; (9) semi-landmark midway between 8 and 10; (10) posterior insertion of anal fin; (11) semi-landmark midway between 10 and 12; (12) semi-landmark midway between 11 and 13; (13) anterior extent of the eye orbit; (14) semi-landmark midway between 12 and 4. Landmarks 1, 6 and 12 were used to allow digital unbending of the specimens (tpsUtil;Rohlf n.d.). We used generalized Procrustes analysis to remove all non-shape variation for each fish and to generate affine and non-affine shape variables (**W** matrix; Mitteroecker and Gunz 2009). We summarized shape variation from the **W** matrix using a principal components analysis to generate relative warps in the package Geomorph (Adams et al. 2017) in R programming software (R Core Development Team 2010; Adams et al. 2017). The fourteen original landmarks yielded 24 relative warps. To account for the reduced dimensionality from the use of sliding semi-landmarks, and to avoid including shape variables that explain only minute amounts of shape variations, we used the first ten relative warps for subsequent analysis,

which combined accounted for >95% of observed shape variation. To characterize body shape variation among these four *Poeciliopsis* species, given that there is no previous data on body shape that can inform our study, we plotted the means and confidence intervals for each species at each location on the first two relative warps (these two relative warps accounted for 66% of total shape variation observed). These plots showed two locations that appear to be outliers (location 11 for *P. presidionis* and location 14 for *P. viriosa*; Fig. 2.2). These locations may have inordinately large effects on patterns of body shape. Thus, we conducted all analyses, including the generalized Procrustes analysis, with and without these two locations. Both sets of analyses yielded the same interpretation, so we present here the results from the analyses that include all locations.

Statistical analyses

We analyzed the data using a mixed-model multivariate analysis of variance (MANOVA) in SAS, version 9.2 (SAS Institute 2008). Given that relative warps are orthogonal and ordered according to the amount of variation they explain, they can be treated as repeated measures with the use of an index variable, analogous to time in a traditional repeated-measures analysis (Scott and Johnson 2010; Wesner et al. 2011*a*; Ingley et al. 2014). We created the index variable by using the identifying order number of the relative warps (i.e. 1 to 10), which was included in the repeated statement for the mixed-model analyses. In all of our analyses, the interaction between main effects and the index variable is the most direct test of our question because the interaction with the index variable tests for differences in shape on each relative warp independently (Wesner et al. 2011*b*; Ingley et al. 2014; Meyers and Belk 2014).

We conducted separate MANOVAS for each species, and in each test, the main effect was the number of co-occurring competitors. Due to differences in the number of locations for each of the species in this study, we coded number of competitors in two different ways to allow replicates for each competition level. First, we designated number of competitors as zero, one, or two plus (meaning two or more competing species); and second, we designated number of competitors as zero or one plus (meaning one or more competing species). For *P. prolifica* and *P. latidens,* we used two separate models utilizing each of the numbering schemes for competition. For *P. viriosa* we were only able to analyze the model with the simpler code (zero competitors and one plus) as the main effect due to number of sampled locations (Table 2.1). Unfortunately, due to the lack of allopatric localities (zero competitors) for *P. presidionis,* we were unable to conduct a formal test of number of competitors for this species (Table 2.1). However, we did include *P. presidionis* samples for general shape comparison in other analysis to show its position in shape space.

We included in all MANOVAS canopy cover and stream slope as covariates. Canopy cover has been used previously as a proxy for resource availability (Johnson 2002), so its inclusion could account for differences in resource availability that may affect body shape (Langerhans and Reznick 2010; Scharnweber et al. 2013). Moreover, higher canopy cover has been shown to be correlated with lower resources in streams that are similar to ours (Grether et al. 2001). Higher resources can lead to a more distended abdomen in poeciliid fishes (Olsson et al. 2007). We used stream slope as a proxy for stream velocity (Zúñiga-Vega et al. 2007; Meyers and Belk 2014). Stream velocity is known to affect body shape in poeciliids, with higher flowing streams causing a more streamlined body shape (Langerhans et al. 2004; Zúñiga-Vega et al. 2007; Langerhans 2008; 2009; Langerhans and Reznick 2010; Ingley et al. 2014). No other

environmental covariates were included because they have no known direct relevance for body shape. However, our statistical test include the first 10 relative warps (as described above).

To visualize the differences in body shape for all analyses, we plotted LS means (± CI) on the first two relative warps axes, which accounted for more than half of the shape variation (65.68%). We also generated thin-plate spline visualizations (Zelditch et al. 2012) to represent the observed changes in body shape and to aid with the interpretation of the differences found for number of competitors.

Results

We found high overlap in body shape among all four species across all locations (Fig. 2). When we removed the two outlier locations, then the overlap in shape space becomes even more extreme. Three of the four species show complete overlap with the other species in shape space as seen on the first two relative warps (Fig. 2.2). Yet, despite this overlap among species, variation within species and locations was quite broad.

Each species showed a shift in body shape when it occurred with competitors. For both *P. prolifica* and *P. latidens* there was a significant effect of the interaction between the index variable and number of competitors (coded as both 0, 1, or 2+, or as 0, 1+). Both canopy cover and stream gradient also showed significant effects on body shape (Table 2.3, Table 2.4). For both *P. latidens* and *P. prolifica,* body shape converged in the presence of competitors (Fig. 2.3). Overall, the body shape of both *P. prolifica* and *P. latidens* when they co-occur with other species is less streamlined and more robust (Fig. 2.4) than when they occur alone. However, some differences in body shape depended on if they co-occured with one or two plus competitors

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(Fig. 2.4). When *P. prolifica* and *P. latidens* co-occured with one competitor they had a more robust shape with a deep belly, more dorsally oriented mouth, a smaller head and eye as opposed to their allopatric shape. However, when they co-occur with two plus competitors the belly shape was intermediate between zero and one competitor, but their eye was larger than the allopatric shape (Fig. 2.4).

Poeciliopsis viriosa also showed a shift in body shape when it occurred with competitors. We also found a significant effect of the interaction between the index variable and number of competitors for *P. viriosa* after accounting for environmental differences. Additionally, the significant effect remained for *P. latidens* and *P. prolifica* when we compared between zero competitors and one plus competitors (Table 2.4). Similar to previous results, we found that when co-occurring with a competitor, all three species converged in body shape (Fig. 2.5). Body shape for all species when co-occurring with one competitor was more robust with a deeper belly, larger eye, less pronounced snout and more dorsally oriented mouth than when no competitor was present (Fig. 2.6).

It was also apparent from the LS means and confidence intervals that not all species responded to competition with the same strength (Fig. 2.3 and Fig. 2.5). If we consider the body shape without competition as a baseline, *P. prolifica* had a greater mean shift in body shape than *P. latidens,* which also shifted more in mean body shape than *P. viriosa* (Fig. 2.3 and Fig. 2.5). This order of the degree of shift in body shape remained even when the outlier locations were removed; although there were some differences, especially for *P. viriosa*, which had smaller degree of body shape shift from its allopatric (zero competitors) body shape (Fig. 2.7). Interestingly, *P. prolifica* had a greater degree of change in body shape when it co-occurred with one competitor, than when it co-occurred with two plus competitors (Fig. 2.3).

Discussion

Suprisingly, we found that all species converge in body shape when they occurred with potential competitors. Convergence is uncommon in studies of character displacement (Grant 1972; Pfennig and Pfennig 2012*c*) and is usually explained by shared environmental effects (Verde Arregoitia et al. 2018). However, the convergence we observed in these *Poeciliopsis* species remained even after accounting for potential environmental drivers of body shape (resource availability and stream velocity). Thus, the pattern appears to be driven by the co-occurrence of competitors, a phenomenon known as convergent character displacement. Furthermore, the shift in body shape that we observed is not consistent with the expected effects of either resources or stream velocity. For example, we found that fish from populations with two plus competitors had a less distended abdomen, even though these sites tended to have a more open canopy cover (more resources) which should lead to a more distended abdomen (Olsson et al. 2007).

Theory suggests that the pattern of convergence we found is consistent with a niche that is saturated with insufficient space to allow species to diverge (Hubbell 2006; Scheffer and van Nes 2006; terHorst et al. 2010; 2018). Interestingly, the convergence in shape we found was not consistently in the same direction as number of competitors increased, meaning that when a species occurred with two or more competitors, we did not find a more extreme shape from when that species only occurred with one competitor. Instead, when two plus competitors co-occurred, we found a body shape that was intermediate to the shape found with one or no competitors. This intermediate shape matched the result of a previous study with multiple competing species

wherein species with multiple competitors tended to have intermediate competitive phenotypes (Miller et al. 2014). It is possible that in multispecies communities, pairwise interactions independent of other species are rare, simply because species interact with several competitors and phenotypically respond with phenotypes suited to deal with all of these competitors (Connell 1980; Strauss et al. 2005). Thus, the multiplicity of interactions could limit adaptation to any particular species, instead resulting in a compromised phenotype suited to dealing with multiple competitors.

Body shape in fishes is known to be affected by several biotic and abiotic factors (Langerhans and Reznick 2010). Each of those factors can create specific morphological responses in fish (Langerhans and Reznick 2010). So what does the body shape found in our study tell us about these three *Poeciliopsis* species? In both *P. latidens* and *P. prolifica* we found that when co-occurring with one competitor they had a deeper body, and a shorter and wider caudal peduncle. We also found that the eye was smaller and the mouth seemed more dorsally located. This shape, in general, doesn't match predictions of benthic vs. limnetic phenotypes (Robinson and Wilson 1995; Ruehl and DeWitt 2005; Palkovacs et al. 2011), lower resources (Olsson et al. 2007), or higher stream velocities (Langerhans et al. 2004; Langerhans 2008; 2009; Ingley et al. 2014). However, all variables (except for the size of the eye) do match body shape due to higher consumption of resources (Palkovacs et al. 2011). Furthermore, when both *P. latidens* and *P. prolifica* co-occur with two plus competitors, the only changes are a larger eye and a shallower body, but the body still remained deeper than the body shape when no competitors co-occur—this result is also consistent with a higher consumption of resources. These differences in body shape were also found in *P. viriosa* when the comparison was only between no competitors and one competitor. Thus, these results all suggest that competition

could be driving body shape to allow individuals to consume more resources. Future studies that focus on head morphology, particularly on mouth characteristics that can be affected by resource consumption, as well as by particular type of food items that these fishes consume should yield further insight into this hypothesis (Tobler 2008; Palkovacs et al. 2011).

Finally, the asymmetric degree of displacement in body shape found here suggests that each species could be affected differently by competition, which could explain the observed differences in degree of shape response. Asymmetries in character displacement are usually posited to be due to differences in competitive abilities among the species considered (Pfennig and Pfennig 2012*a*). It is possible that the greater shift of *P. prolifica* when co-occurring with one competitor, as opposed to when it co-occurs with two plus, is due to the effects of the presence of other competitors that may lower the competitive effect of each species on *P. prolifica* (maybe through a non-hierarchical interaction). Another possibility is that each species has a species-specific effect, and that the degree of response depends on which species is co-occurring. However, future studies that focus on competitive abilities and each pairwise interaction, as well as different combinations of multispecies interactions, will be needed to more fully explore the causes of these asymmetries. Future studies could also consider laboratory tests of performance in food consumption of each species from the different competitive levels. This may shine some light on the intermediate shape of *P. prolifica* and *P. latidens* when co-occurring with two or more competitors. Furthermore, these results could strengthen the argument that when more competitors coexist, responses will be intermediate to account for multispecies interactions, instead of pairwise interaction.

One caveat is that we cannot exclude the possible effects of correlated selection on other traits, most notably life history (Langerhans and Reznick 2010). Studies have shown that

coexistence can be maintained in competitive environments through trade-offs with life history traits (Hutchinson 1957; Levine and Rees 2002; Leibold et al. 2004; Calcagno et al. 2006; Chapuis et al. 2017). Life history can contribute to differences in body shape in poeciliids (Plaut 2002). In particular, a deeper body with a larger belly could be the byproduct of adaptation to a large reproductive investment (Plaut 2002; Ghalambor et al. 2004; Langerhans and Reznick 2010). Nevertheless, all species included in this study have superfetation (i.e. the ability to carry multiple broods simultaneously), which has been proposed as an adaptation to maximize reproductive investment without a trade-off in morphology (Zúñiga-Vega et al. 2007; 2010). Furthermore, reproductive allocation itself can be molded by tradeoffs between selection for reproduction and mobility required for foraging (Ghalambor et al. 2003; 2004). However, if there is an effect of life history on the body depth, it would not discount the effects found here pointing to competition as a driver of body shape.

Like several studies of evolutionary change of body shape, we assume that differences in body shape are underpinned, at least to some degree, by genetic changes among populations. Although we did not directly test this, and cannot exclude the possibility of plasticity, shape is typically driven to some extent by heritable variation in poeciliid fishes (Reznick et al. 2008). Moreover, studies on female guppies (closely related to our species) have failed to induced a plastic response on body shape due to different levels of resource availability, where such responses did occur in male guppies (Robinson and Wilson 1995).

Conclusion

We found surprising evidence for convergent character displacement in body shape in three species of *Poeciliopsis*. Overall, our results suggest that competition in multispecies interactions is more complex than in simple pairwise comparisons. The important conclusion from our work is that when multiple competing species co-occur, simply considering pairwise competitive interactions to explore trait evolution is likely insufficient. In our work, we found a non-intuitive pattern of trait convergence that makes best sense only in the context of multiple competing species.

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Table 2.1: Sample sizes and locations for each of the four *Poeciliopsis* species.

Species	Number of Competitors	Sample Size	Locations ID
P. prolifica			
	0	177	1,2
	1	329	7,8,9,10,11,12,13
	2+	414	14,15,17
			7,8,9,10,11,12,13,14,1
	1+	743	5,17
	0	83	5,6
P. latidens			
	1	229	7,8,9,10
	2+	81	14,16,17
	1+	310	7,8,9,10,14,16,17
P. viriosa			
	0	49	3,4
	1+	175	13,14,16,17
P. presidionis			
	1+	94	11,12,16,17

Table 2.2: Environmental characteristics of the sampled locations.

Locations ID	Canopy cover (%)	Str. slope	Temperature (° C)	pH	Conductivity (u/s)
1	—	22.5	23.3	6.96	157
2	69	0	25.7	7.41	—
3	47	3.5	26.5	7.16	226
4	62	4	27.4	8.22	—
5	100	1.5	28.5	8.08	464
6	98	0	25.1	7.65	228
7	41	2.5	30	7.04	479
8	—	3.5	28.2	7.34	392
9	100	5.5	31.1	7.82	257
10	82	4.5	30	8.02	1082
11	100	3	24.8	7.73	1186
12	100	0	26.9	8.42	—
13	69	7	27	8.3	—
14	100	7.5	27.5	8.09	134
15	36	1	28.2	7.43	—
16	75	4	29.1	8.03	161
17	37	9	30.1	8.13	299

Dash indicates missing data.

Table 2.3: Multivariate analysis of covariance for body shape in *P. prolifica* and *P. latidens* for number of competitors coded as zero, one and two plus (see Methods for an explanation of these categories).

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Species	Effect	DF	F value	P value
P. prolifica				
	Index	9,3107	54.55	<.0001**
	Num. of competitors	2,2675	1.23	0.2919
	Stream slope	1,2675	5.45	0.0196*
	Canopy	1,2675	7.66	0.0057*
	Num. of competitors x index	18,423 3	22.22	<.0001**
	Canopy x index	9,3107	31.86	<.0001**
	Slope x index	9,3107	29.44	<.0001**
P. latidens				
	Index	9,1275	27.83	<.0001**
	Num. of competitors	2,1024	0.9	0.4087
	Stream slope	1,1024	6.21	0.0129*
	Canopy	1,1024	0.7	0.4022
	Num. of competitors x index	18,173 5	22.43	<.0001**
	Canopy x index	9,1275	16.95	<.0001**
	Slope x index	9,1275	11.93	<.0001**

Table 2.4: Multivariate analysis of covariance for body shape in *P. prolifica*, *P. latidens* and *P. viriosa* for number of competitors coded as zero and one plus (see Methods for an explanation of these categories).

Species	Effect	DF	F value	P value
P. prolifica				
	Index	9,3107	57.59	<.0001**
	Num. of competitors	1,2619	2.23	0.1352
	Stream slope	1,2619	6.55	0.0106*
	Canopy	1,2619	9.71	0.0019*
	Num. of competitors x index	9,3107	16.24	<.0001**
	Canopy x index	9,3107	37.63	<.0001**
	Slope x index	9,3107	45.15	<.0001**
P. latidens				
	Index	9,1275	24.8	<.0001**
	Num. of competitors	1,1005	0.45	0.5002
	Stream slope	1,1005	0.98	0.323
	Canopy	1,1005	5.5	0.0192*
	Num. of competitors x index	9,1275	28.13	<.0001**
	Canopy x index	9,1275	18.56	<.0001**
	Slope x index	9,1275	25.41	<.0001**
P. viriosa				
	Index	9,899	19.15	<.0001**
	Num. of competitors	1,989	22.89	<.0001**
	Stream slope	1,989	43.59	<.0001**
	Canopy	1,989	247.05	<.0001**
	Num. of competitors x index	9,899	8.33	<.0001**
	Canopy x index	9,899	33.9	<.0001**
	Slope x index	9,899	8.04	<.0001**

Figure 2.1: Map showing sampled locations. Symbols correspond to circle- no competitor, triangle – one competitors, square- two plus competitors. Colors correspond to: blue-*P. latidens*, red-*P. prolifica*, and green-*P. viriosa*. Numbers in each location corresponds to location ID (found in Table 2.1).
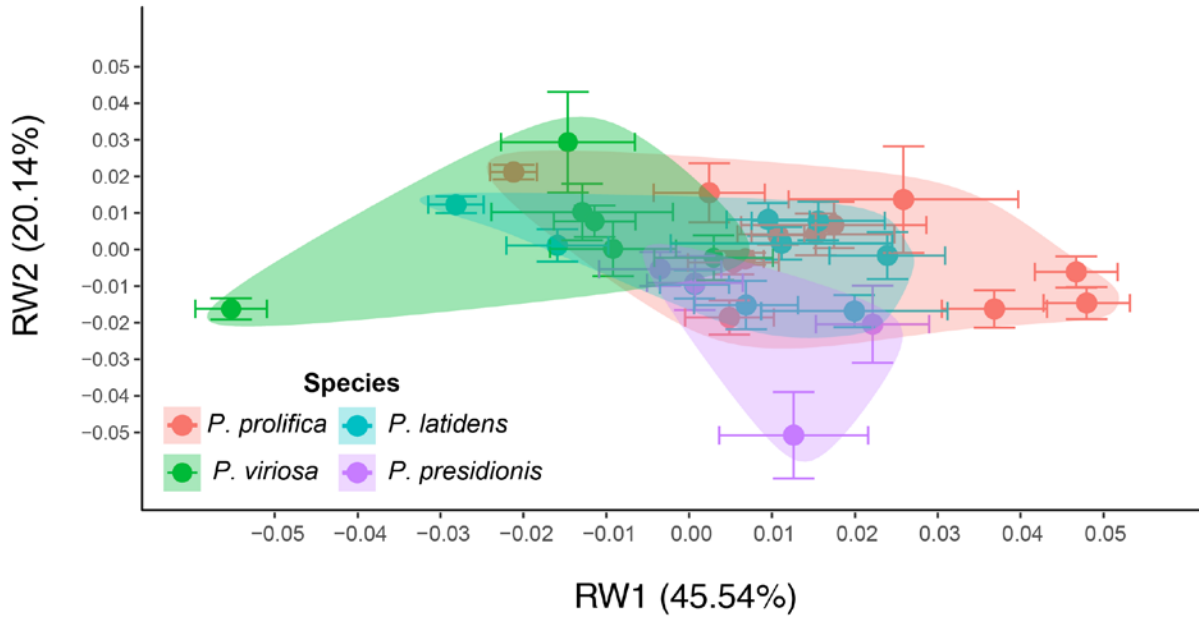
Figure 2.2: Means of relative warps scores (±CI) for each species by location. Colors correspond to: blue-*P. latidens*, red-*P. prolifica,* green-*P. viriosa,* and purple-*P. presidionis.*
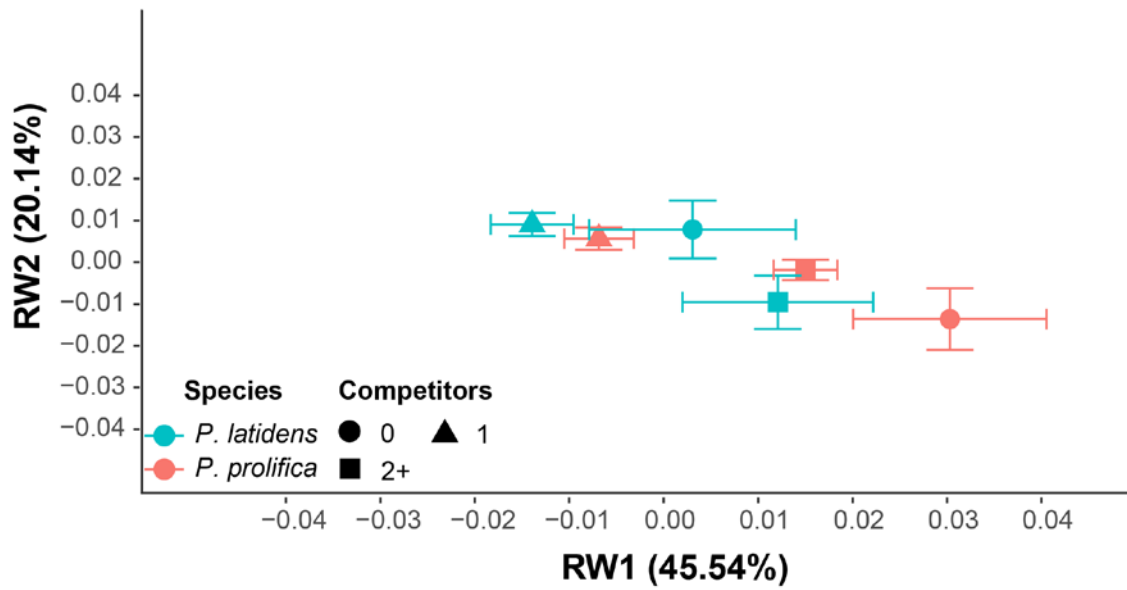
Figure 2.3: Least squares means of relative warps (RW) scores (±CI) for number of competitors (0,1,2+) for *P. latidens* and *P. prolifica*. Symbols correspond to: circle- no competitors, triangle- one competitor, and square- two plus competitors. Colors correspond to: blue-*P. latidens* and red-*P. prolifica*.
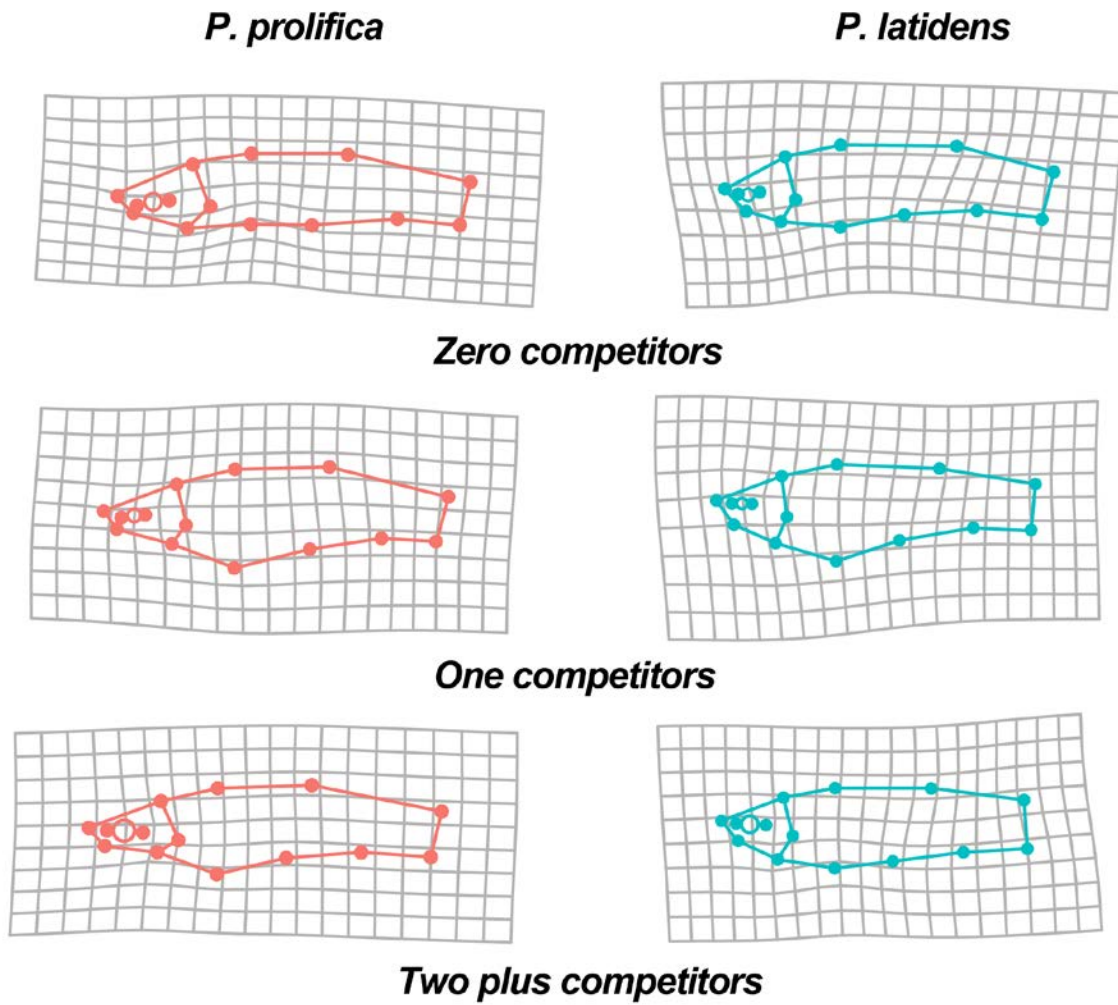
Figure 2.4: Body shape for *P. latidens* and *P. prolifica* for the different number of competitors (0,1,2+). Thin-plate splines grids at 3x scale with added lines to assist interpretation. Colors correspond to: blue-*P. latidens*, and red-*P. prolifica.*
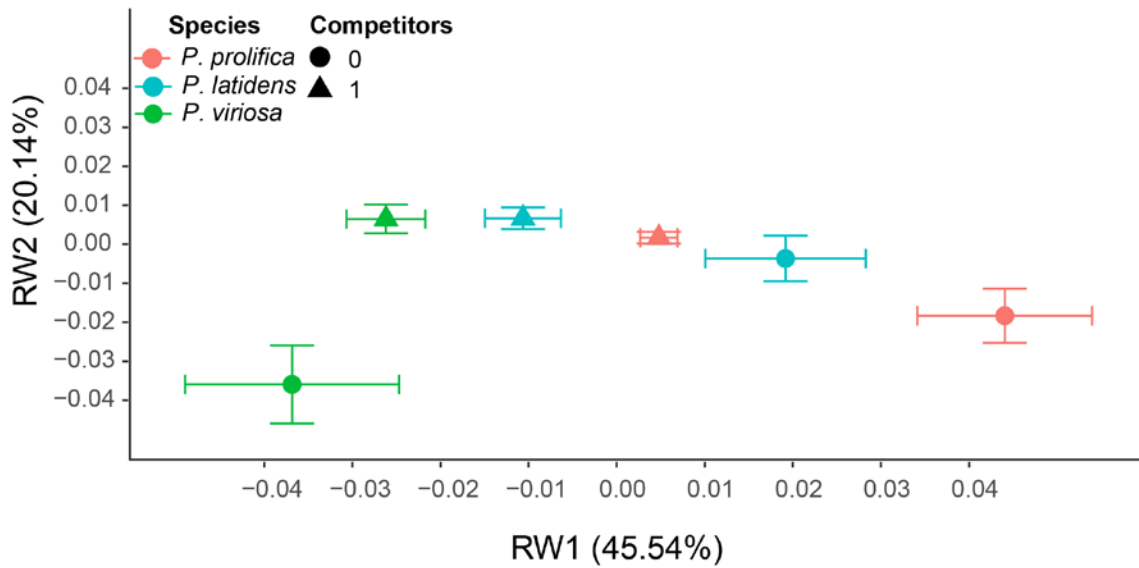
Figure 2.5: Least squares means of relative warps (RW) scores (±CI) for number of competitors (0,1+) for *P. latidens*, *P. prolifica* and *P. viriosa*. Symbols correspond to: circle- no competitors, triangle- one competitor, and square- two plus competitors. Colors correspond to: blue-*P. latidens*, red-*P. prolifica,* and green-*P. viriosa.*
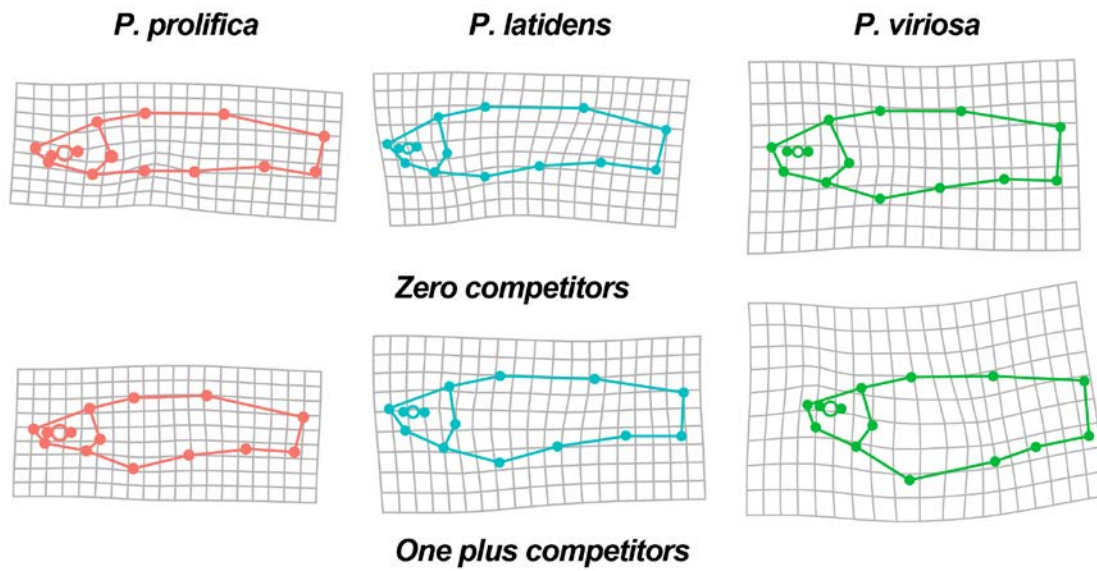
Figure 2.6: Body shape for *P. latidens*, *P. prolifica* and *P.viriosa* for the different number of competitors (0,1+). Thin-plate splines grids at 3x scale with lines added to assist interpretation. Colors correspond to: blue-*P. latidens*, red-*P. prolifica,* and green-*P. viriosa.*
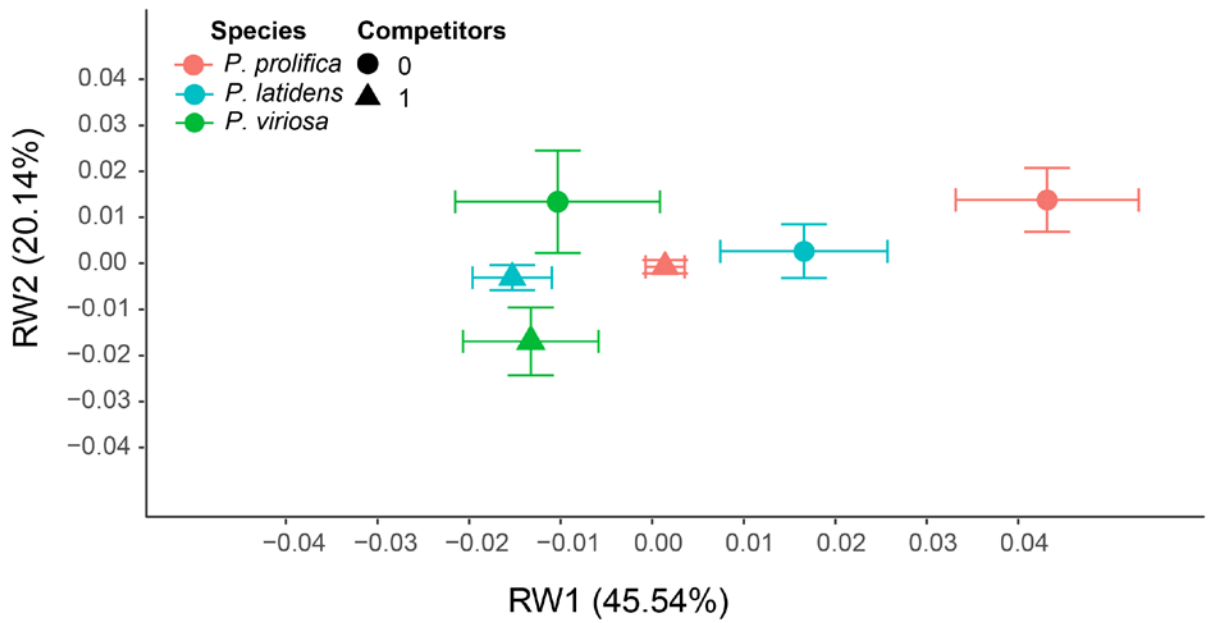
Figure 2.7: Least squares means of relative warps (RW) scores (±CI) for number of competitors (0,1+) for *P. latidens*, *P. prolifica* and *P. viriosa* without potent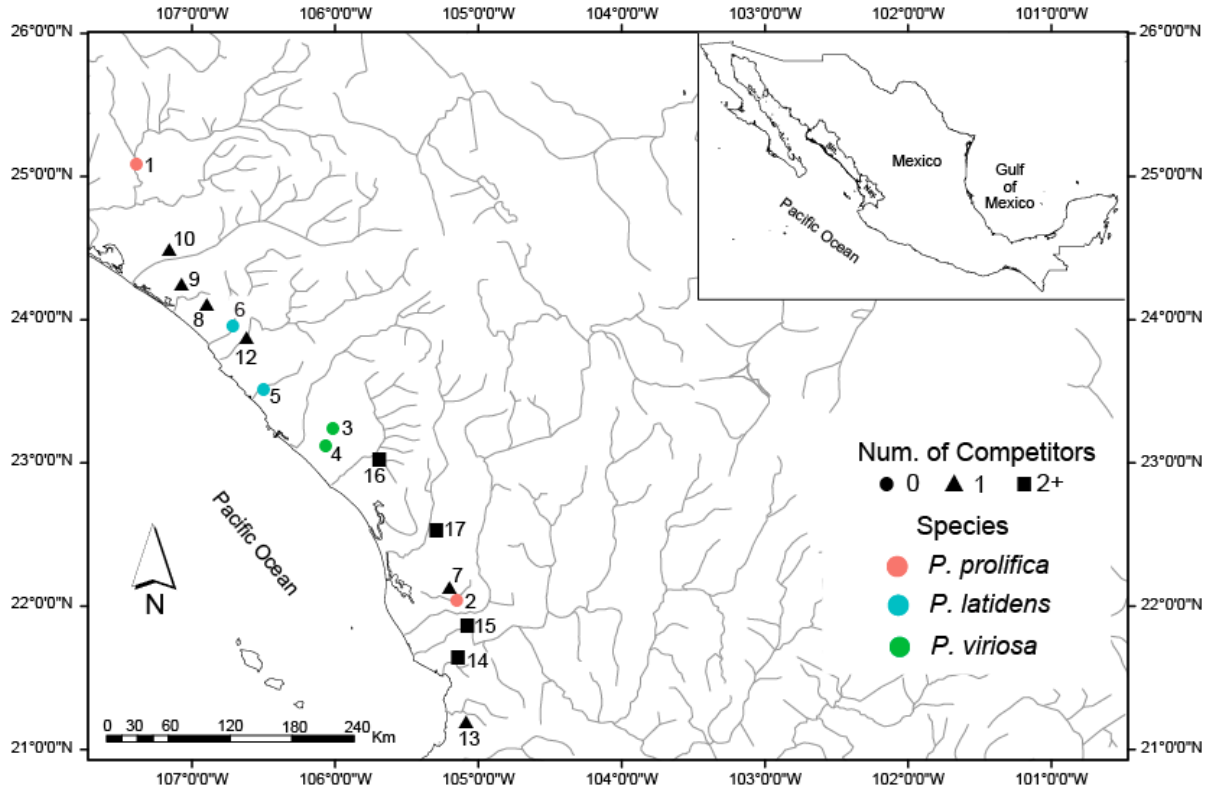ial outlier locations. Symbols correspond to: circle- no competitors, triangle- one competitor, and square- two plus competitors. Colors correspond to: blue-*P. latidens*, red-*P. prolifica,* and green-*P. viriosa.*

Chapter 3

What drives life history in the livebearing fish *Poeciliopsis prolifica*? An assessment of four

putative selective agents

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Abstract

Life history traits are directly linked to fitness, and therefore, can be highly adaptive. Moreover life history traits can be shaped by several different selective pressures, including density, predation, and resource levels. However, these selective pressures are usually considered independently and we lack a clear understanding of how they interact in shaping life history evolution. Here we test the simultaneous effects of several potential selective pressures on life history traits in the livebearing fish *Poeciliopsis prolifica.* We employ a multi-model inference approach. We focus on several known agents of selection, including resource availability, stream velocity, population density, and interspecific competition and their effect on four life history traits (reproductive allocation, superfetation, number of embryos, and individual embryo size). We found that competition, both intraspecific and interspecific, were the most important selective agents for all life history traits, except for embryo size, and had a positive association. For individual embryo size we found that all single agent models were equivalent and it was unclear that which selective agent best explained variation for this life history trait. We also found that models that included density and competition as direct effects were better supported than those that included these factors as indirect effects through their influence on resource availability. Our study underscores the importance of interspecific competitive interactions on shaping life history traits and suggests that these interactions should be considered in future life history studies.

Introduction

Life history traits can be highly adaptive (Roff 2002; Chapuis et al. 2017). Demostrating that natural selection drives life history evolution usually begins with comparative studies of natural populations in the wild (Roff 2002). Such studies have shown that life history traits can evolve in response to several selective pressures, both biotic and abiotic (Johnson and Bagley 2011). Some important selective agents known to affect the evolution of life histories are population density (Reznick et al. 2002; Reznick et al. 2012), predation (Martin 1995; Johnson and Belk 2001; Reznick et al. 2001; Roff 2002; Chapuis et al. 2017), resource availability (Reznick and Yang 1993; Grether et al. 2001; Roff 2002; Pérez-Mendoza et al. 2014; Zani and Stein 2018), elevation (Badyaev and Ghalambor 2001; Johnson and Bagley 2011), and, in the case of aquatic organisms, water velocity (Reznick et al. 2002; Ghalambor et al. 2004; Zúñiga-Vega et al. 2007; Reznick et al. 2012; Banet et al. 2016; Heins and Baker 2017). However, most studies tend to focus on a single factor, although it is clear that life history traits can be affected and shaped simultaneously by several factors (Moore et al. 2016).

Studies that have examined how several factors affect life history traits have shown that life histories can respond in a predictable and repeatable way to certain selective agents, but not to all of them (Moore et al. 2016). For example, in several livebearing fish species predation drives life history evolution in a predictable and repeatable manner (Moore et al. 2016). However, the response to other selective factors can depend on the population studied (Moore et al. 2016). For some populations, it is clear that several pressures may be acting in concert (Johnson 2002; Moore et al. 2016). Nevertheless, we still a lack of studies that allow us to draw general predictions about the occurrence of similar responses to several selective pressures, and which selective pressures have primacy in wild population.

Here, we evaluate several hypotheses about the relative importance of biotic and abiotic factors in shaping life history traits using a multi-model inference approach in a group of livebearing fishes (Johnson and Bagley 2011). Life history evolution is well studied in livebearers (Johnson and Bagley 2011), allowing us to build specific hypotheses of how each factor could affect life history traits. However, some selective agents have only received modest consideration in this type of study (interspecific competition and stream velocity; Johnson and Bagley 2011). Given this modest inclusion it is unknown how important are interspecific competition and stream velocity in comparison to other more commonly studied selective agents. Thus, we model four hypothetical drivers of life-history variation: resource availability, population density, stream velocity, and interspecific competition (see Table 3.1 for hypotheses), each of which is a known agent of life history evolution in livebearing fishes (*Poeciliidae;* Johnson and Bagley 2011). We compare these selective agents to evaluate the importance of each on life histories of *Poeciliopsis prolifica* and test if the effect of each selective agent is in accordance with previous hypotheses (Table 3.1).

Methods

Study system and collection sites

Poeciliopsis prolifica is distributed through northwestern Mexico on the Pacific slope from the Rio Yaqui, Sonora south to near Las Varas, Nayarit (Miller et al. 2005). Populations exist under a variety of environmental conditions that include differences in fish density, stream velocity, fish community structure, and habitat characteristics. This provides a range of selective conditions under which populations might evolve local adaptations. We collected *P. prolifica*

females using hand-held seine nets (1.3 m x 5 m; 8 mm mesh size) from 12 populations (Fig. 1) during the dry season.

For each locality we quantified four environmental parameters. We estimated relative density by calculating the average number of *P. prolifica* fish collected per seining attempt, a value found to be positively correlated with actual density (Johnson 2002). We estimated interspecific competition as the number of co-occurring species of the same genus, as *P. prolifica* can co-occur with up to three species of the same genus: *P. viriosa*, *P. latidens*, and *P. presidionis* (Mateos et al. 2002; Miller et al. 2005). To our knowledge there are no published accounts of the degree of similarity or the niche overlap among these four species. However, general accounts of these four species indicated that they are ecological similar—they all inhabit the mid-water column in streams and small rivers, they are similar in body form, and they are omnivorous, consuming plant and animal matter (Miller et al. 2005). Furthermore we collected these species in the same microhabitat. Hence, we conclude that due to their close phylogenetic relationship and ecological similarity there is potential for competitive interactions. We used number of co-occurring species as an indicator of level of competition in our analysis and refer to it as competition. We measured canopy cover with a hand-held densiometer and we calculated stream slope in ArcMap 10.6 (ESRI; Enviromental Systems Research Institute 2014), as the difference between upper elevation and lower elevation of a 2-km segment of stream for each locality. We found no piscivorous predators in the localities sampled.

Life history traits

We quantified four different life history traits: superfetation (number of simultaneous broods carried by a female); individual embryo size; number of embryos (across all broods; Frías-Alvarez and Zúñiga-Vega 2015); and reproductive allotment (RA). We classified embryo development stage using the 11-point stage scale developed by Haynes (1995). We defined a brood as all the embryos that share the same developmental stage (Haynes 1995). We measured individual embryo size by drying an entire brood of offspring in a desiccating oven for 48 h at 55 °C and dividing the brood dry mass by the number of embryos in the brood. To avoid non-independence in individual embryo size due to the fact that females can have more than one brood, we only considered the brood at the most advance developmental stage of each female for the calculation of individual embryo size. We obtained female somatic dry mass by drying the female soma (minus the intestinal tract and offspring) for 48 h at 55 °C. For RA, we used the total dry mass of all broods of each female relative to somatic dry mass of the female (Tomkins and Simmons 2002).

To generate comparable estimates among populations for each life history trait we adjusted each trait by an analysis of covariance (ANCOVA). We adjusted individual embryo for maternal somatic dry mass and brood developmental stage, to obtain comparable size and stage "free" measurements for analysis. We only adjusted superfetation, number of embryos and RA for maternal somatic dry mass. To meet assumptions of normality for the ANCOVAS, we used the following transformations on the life history traits: superfetation and total number of embryos were squared-root transformed, whereas embryo size and RA were log transformed. Hence, the comparison among populations are based on adjusted least squared means generated by the ANCOVA models that allowed us to compare size-free and stage-free life history traits.

Before using the adjusted life histories in a model selection approach, we tested for population differences in life histories by employing a multivariate analysis of covariance (MANCOVA). Likewise, we included maternal somatic dry mass and stage of development as covariates in the MANCOVA. We found significant differences among populations of *P. prolifica* in all life history traits ($F_{11,1136}=14.75$, $P \leq 0.001$), so we proceeded with the model selection approach. All of these analyses were implemented in R software (R Core Development Team 2010).

Selective agents

We chose to characterize four selective agents: competition, resource availability, stream velocity and population density. All are known to affect life history traits, either directly or indirectly (Johnson and Bagley 2011). We use stream slope to characterize stream velocity, it is known that stream velocity increase as stream slope increases, thus this indirect measure should be a good proxy for stream velocity (Gore and Banning 2017). Likewise, we estimated resource availability by our measure of canopy cover, which is an indicator of primary productivity (Grether et al. 2001). High canopy cover indicates lower primary productivity (Grether et al. 2001). Canopy cover has been found to be a good proxy for resource availability in other studies with streams similar to ours (Grether et al. 2001). Two populations had missing data on canopy cover (localities four and seven); to avoid the exclusion of these in the analysis we used aerial images from Google Earth to calculate percent cover in ImageJ. This approach has been used before and found to be strongly correlated with field measurements of canopy cover (Inskeep et al. 2011). In our localities we also found a strong correlation between our field measurments of

canopy cover and our calculations of canopy cover from aerial images ($R^2 = 0.76$, p = 0.010). To understand if these selective agents covaried, we conducted a pairwise correlation tests. However, we found no significant correlations among these selective agents (Table 3.2).

Model selection

We generated a set of 14 candidate models that represent competing biological hypotheses of the way the four putative selection agents could act to shape life history traits (Fig. 3.2). We did not include all possible interactions between factors because we wanted to include only those that represent plausible life histories hypotheses taken from theory (Table 3.1). We used a structural equation modeling approach (i.e. path analysis) because it allowed us to assess both direct and indirect effects of the selective agents. Assessing indirect effects is important in our study as some selective agents (e.g. competition and density) are usually only considered in life histories literature as acting through indirect effects (Table 1; Scott and Johnson 2010; Reznick et al. 2012). We know that life history traits can co-vary and its theory suggest that it may evolve as an integrated suite of traits (Fisher 1930; Reznick 1985). This assumption is important as it affects whether life histories can be evaluated as a collective strategy or if each life history trait should be considered separately. To assess this assumption we used a confirmatory factor analysis that allowed us to test if a single factor (i.e. a single life history variable) could adequately summarize all four life history traits measured (Burnham and Anderson 2002). During this confirmatory analysis we found a negative error for RA. We determined that the negative error was due to sample variance (Van Driel 1978; Chen et al. 2001), thus we restricted the error to a small positive number (0.01) following Van Driel (1978). Fixing the error term as

a small positive number allowed us to continue running a factor analysis and retain the potential for error in the measurement of RA. We found that three of the four life history traits appear to behave as an integrated suite of traits in *P. prolifica*, as they all had a positive association and loadings greater than 0.50 in the calculated life history factor (Fig. 3.3), corroborating the idea that certain life history traits evolve in an integrated fashion (Fisher 1930; Reznick 1985). In contrast, embryo size had a small loading and very little variation explained by this factor. Thus, our life history factor was not a good reflection of embryo size, so for all the models we treated embryo size separately. We therefore proceeded to test the different hypotheses through a multi-model inference approach with two life histories measures: the life history factor (number of embryos, reproductive allocation, and superfetation) and embryo size alone.

We ran all 14 candidate models in path analysis using the software Amos (Arbuckle 2013). All models were run using a maximum likelihood estimator. For each model, we generated an Akaike Information Criteria score (corrected for small sample sizes; AICc). We used AICc scores to identify models that best fit the data. Models in which AICc scores differs by less than two are generally considered indistinguishable (Burnham and Anderson 2002; Burnham et al. 2011). We also calculated the model average standard total effect for each selective agent using all 14 models and their associated AIC weights.

Results

Density and competition were the best predictors of number of embryos, RA, and superfetation in *P. prolifica*. We found that for the life history factor, models with both density and competition as direct effects had lower AICc scores than all other models. However, it was

not possible to distinguish between density and competition as they differed by less than two AIC score units (Table 3.3). For embryo size, all models that included a single selective agent had the lowest AICc values and were equivalent in terms of AICc (Table 3.4). This suggests that for *P. prolifica* variation in some of the life history traits is better explained by density and competition than by resource availability or stream velocity, whereas several selective pressures are equally likely to be shaping embryo size.

Although both density and competition were undistinguishable based on AICc scores for the life history factor, they differed in the strength of their effects on life histories. Density had a stronger effect on life histories than competition (Figure 3.4), but both had a positive association. In other words, increasing density or competition results in an increase in all three life history traits (number of embryos, RA, superfetation).

For embryo size, all selective pressures had the predicted effect from theory (Table 3.1; Figure 3.5). Embryo size increased as density increased, and decreased as stream gradient and resources increased. In contrast competition had an unexpected positive effect on embryo size, instead of a negative effect that is expected due to trade-offs in life history response (Table 3.1). This said, the model average standard total effect was generally low, suggesting the strength of these selective pressures was overall somewhat weak in terms of the effect on embryo size (Figure 3.5).

When comparing models with only direct effects and only indirect effects at comparable nesting levels (models 5 vs. 6, 8 vs. 9, and 11 vs. 12), we found that most are not distinguishable from one another (Table 3.3 and Table 3.4). In other words, models with direct or indirect were comparable in their ability to explain variation in life history.

Discussion

We found that simpler models with a single putative selective agent better fit predicted life history traits than more complex models with two or more factors. In other words, the inclusion of additional selective agents did not explain sufficient additional variation in life histories to offset the penalty for additional parameters in the model (Burnham and Anderson 2002; Anderson 2008; Burnham et al. 2011). Johnson (2002) found a similar pattern when exploring life history variation in the fish *Brachyrhaphis rhabdophora*. In that study, the selective agents were indistinguishable in their ability to predict life history. However, in our study we found that for three of the four life history traits studied, competition and density best explained variation in *P. prolifica* life history.

As competition increased, we found an increase in number of embryos, RA, and superfetation. These results are consistent with theoretical predictions when *P. prolifica* has lower competitive abilities but is better at reproduction (Hutchinson 1957; Levine and Rees 2002; Leibold et al. 2004; Kneitel and Chase 2004; Calcagno et al. 2006; Chapuis et al. 2017). However, we did not measure competitive abilities which will be needed to confirm this trade-off between reproduction and competitive abilities (Kneitel and Chase 2004; Calcagno et al. 2006). Nevertheless, a trade-off is the most likely explanation for the observed positive association between these life history traits and competition, otherwise a decrease on number of embryos and RA is expected (Scott and Johnson 2010). Furthermore, competition was also positively associated with embryo size, meaning that for *P. prolifica* an increase in competition causes an increase in all four life history traits, which can only be possible if there is a trade-off

between competitive ability and reproduction. Additionally, when competition was included in the model as an indirect effect on resources number of embryos, RA, and superfetation reduce as competition increased, which is consistent with theoretical predictions (Bashey 2008; Johnson and Bagley 2011), lending further support to the possibility of *P. prolifica* having lower competitive abilities but being better at reproduction.

Density was also positively associated with each of the life history traits evaluated here (RA, number of embryos, superfetation, and embryo size). These results are somewhat puzzling in that increasing density is expected to result in a decrease in number of embryos and in reproductive allocation (Johnson and Bagley 2011; Moore et al. 2016). Higher density should result in a reduction in per capita resource availability, resulting in lower RA and number of embryos, but a higher superfetation and larger embryo size (Table 3.1). Two possibilities could account for our observations. First, competition in our system may not be through competition for available food resources as appears to be the case in other studies (Reznick 1989; Reznick et al. 2002). In other words, life history traits in *P. prolifica* may be shifting to improve competitive ability in a context different from competition for resources, such as microhabitat (Table 3.1). Second, most studies examining the effects of density on life history have been conducted in species that lack superfetation (e.g. Smith 2007; Reznick et al. 2012). The ability to carry multiple broods simultaneously in *P. prolifica* might confer advantages that alter the effect of density on life history (Zúñiga-Vega et al. 2017). For example, higher superfetation has been related to higher reproductive allocation in other livebearing fishes (Zúñiga-Vega et al. 2017), and it has been suggested that superfetation allows these species to overcome morphological constraints in terms of reproductive investment (Frías-Alvarez and Zúñiga-Vega 2015; Zúñiga-Vega et al. 2017). Thus, it is possible that species that superfetate can have large

offspring without the typical trade-off in terms of number of offspring (Olivera-Tlahuel et al. 2015). This could allow for the positive relationship between density and the life history traits observed in our study.

The effect of all selective pressures on embryo size were small in *P. prolifica*. It is unclear why this is the case. It may be that in general for *P. prolifica* there is little variation in embryo size. It is also possible that the selective agents that we examined here are simply weak in their ability to shape embryo size. Despite the modest responses of offspring size to the putative selective agents, the pattern of change observed here was consistent in the direction predicted by theory (Table 3.1).

We found that the effect of competition was generally weaker than the effect of density in predicting life history. This supports a body of theory that argues that intraspecific competition in general should be stronger than interspecific competition (Chesson 2000; Chesson 2013; Adler et al. 2018). Although this was true for the majority of life history traits examined here, this was not the case for embryo size where competition between species was stronger than the effects of density, a measure of intraspecific competition. We note that studies which consider interspecific competition as a selective pressure in life histories are uncommon (Scott and Johnson 2010; Chapuis et al. 2017). Hence, our findings point to a promising area for future research, especially in understanding the interplay between, within, and among species interactions.

Finally, it is interesting to note that both density and competition best explained our data when these factors were included as direct effects on life histories. Usually, when competition (both intraspecific and interspecific) is considered in life history research, it is usually explained

in terms of its indirect effect on life history mediate through food resource availability (Reznick and Yang 1993; Scott and Johnson 2010; Johnson and Bagley 2011; Wilson 2013). However, this was not the case in our study. Several possibilities could account for this result. First, food resource availability may not be as important in our system as others; and it is possible that competition could be acting through some other environmental variable, such as habitat use. Second, this could simply be an artifact of the model selection framework used in our analysis. Models with indirect effects required more parameters, and consequently these models are penalized for the inclusion of additional parameters. Hence, the models may not be favored not because indirect effect are unimportant, but because they are not sufficiently important to offset the const of including them in the model. Finally, it is possible that competition indeed has a strong direct effect on life history in *P. prolifica*. If this is the case, we need to consider how competition could act as a direct selective agent on life histories, an area where we still lack a strong theoretical foundation to make predictions.

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Table 3.1: Potential direct and indirect effects of the four putative selective agents on life histories of *Poeciliopsis prolifica*. Numbers in the path correspond to the numbers in the path graph global model (see Figure 3.2).

Selective Agent	Path	Effect on life history	Prediction	Rationale	Reference
Resources (R)	1	Direct	An increase in resources will result on an increase in num. of embryos and RA, but a decrease in superfetation and embryo size.	Higher resources should result in greater body condition and thus greater investment in reproduction.	(Reznick and Yang 1993; Johnson and Bagley 2011)
Density (D)	2	Direct	An increase in density will result on an increase in superfetation and embryo size, but a decrease in num. of embryos and RA.	Higher density may increase competition for other environmental factors, such as habitat and not through resource availability. Competition should still lower body condition and result in lower investment in reproduction.	(Johnson and Bagley 2011)
	5	Indirect through resources	An increase in density will result on an increase in superfetation and embryo size, but a decrease in num. of embryos and RA.	Higher density increases competition for resources resulting in lower resources per individual, which causes lower body condition and lower investment in reproduction.	(Reznick et al. 2002; Johnson and Bagley 2011; Reznick et al. 2012)
Competition (C)	3	Direct	An increase in competition will result on an increase in superfetation, num. of embryos and RA, but a decrease in embryo size.	Higher interspecies competition causes a pressure for higher reproduction to compete and coexist. LH acts as a tradeoff for competition that allows coexistence. Simply said there is a trade-off between competitive abilities and reproduction.	(Hutchinson 1957; Levine and Rees 2002; Leibold et al. 2004; Kneitel and Chase 2004; Calcagno et al. 2006; Chapuis et al. 2017)
	6	Indirect trough resources.	An increase in density will result on an increase in superfetation and embryo size, but a decrease in num. of embryos and RA.	Higher interspecies competition increases overall density that decreases resources. As resources are lower per individual this causes low body condition and lower investment in reproduction.	(Scott and Johnson 2010; Wilson 2013)
Stream Velocity (S)	7	Direct	An increase in stream velocity will result on an increase in superfetation, num. of embryos and RA, but a decrease in embryo size.	Higher flow environments result in more streamlined body shapes that should increase superfetation, allowing reproductive effort to be higher or at least unchanged.	(Zúñiga-Vega et al. 2007; Zúñiga-Vega et al. 2010; Johnson and Bagley 2011)

Table 3.2: Pairwise correlation coefficients with P values of the four putative selective agents.

Environmental variable	Competition	Resources	Stream Slope	Density
Competition		0.20	0.67	0.53
Resources	-0.39		0.79	0.27
Stream Slope	-0.14	0.09		0.23
Density	-0.20	-0.35	0.37	

Values below the diagonal are correlation coefficients, above the diagonal P values for the corresponding correlations.

Table 3.3: List of the a priori 17 candidate models of the effect of the four putative selective agents on life history with corresponding AICc values, ΔAICc, and AICc weights (w).

Selective agents	Model	Paths	AICc	ΔAICc	w	w cum.
D	2	2,7	31.08	0	0.416	0.416
C	3	3,7	31.37	0.29	0.360	0.775
S	4	4,7	33.29	2.21	0.138	0.913
R	1	1,7	34.22	3.14	0.086	0.999
RC	9	1,6,7	45.92	14.84	<0.001	0.999
RD	5	1,2,7	46.91	15.83	<0.001	0.999
RC	8	1,3,7	47.33	16.25	<0.001	0.999
RC	10	1,3,6,7	48.95	17.87	<0.001	0.999
RD	6	1,5,7	49.02	17.94	<0.001	0.999
RD	7	1,2,5,7	49.06	17.98	<0.001	1.000
RCD	11	1,2,3,7	60.97	29.89	<0.001	1.000
RCD	12	1-5-6-7	63.18	32.1	<0.001	1.000
RCD	13	1,2,3,5,6,7	63.38	32.3	<0.001	1.000
RCDS	14	1,2,3,4,5,6,7	86.03	54.95	<0.001	1.000

Selective agents are abbreviated as follows: R= resources, D= density, C= competition, S= stream velocity.

Table 3.4: List of the a priori 14 candidate models of the effect of the four putative selective agents on embryo size with corresponding AICc values, ΔAICc, and AICc weights (w).

Selective agents	Model	Paths	AICc	ΔAICc	w	w cum.
R	1	1,7	10.46	0	0.245	0.245
D	2	2,7	10.46	0	0.245	0.491
C	3	3,7	10.46	0	0.245	0.736
S	4	4,7	10.46	0	0.245	0.981
RD	6	1,5,7	17.43	6.97	0.008	0.989
RD	5	1,2,7	18.76	8.3	0.004	0.993
RC	8	1,3,7	19.29	8.83	0.003	0.996
RC	9	1,6,7	20.35	9.89	0.002	0.997
RD	7	1,2,5,7	20.91	10.45	0.001	0.999
RC	10	1,3,6,7	20.91	10.45	0.001	0.999
RCD	12	1,5,6,7	28.42	17.96	<0.001	1.000
RCD	11	1,2,3,7	29.43	18.97	<0.001	1.000
RCD	13	1,2,3,5,6,7	31.84	21.38	<0.001	1.000
RCDS	14	1,2,3,4,5,6,7	40.77	30.31	<0.001	1.000

Selective agents are abbreviated as follows: R= resources, D= density, C= competition, S= stream velocity.
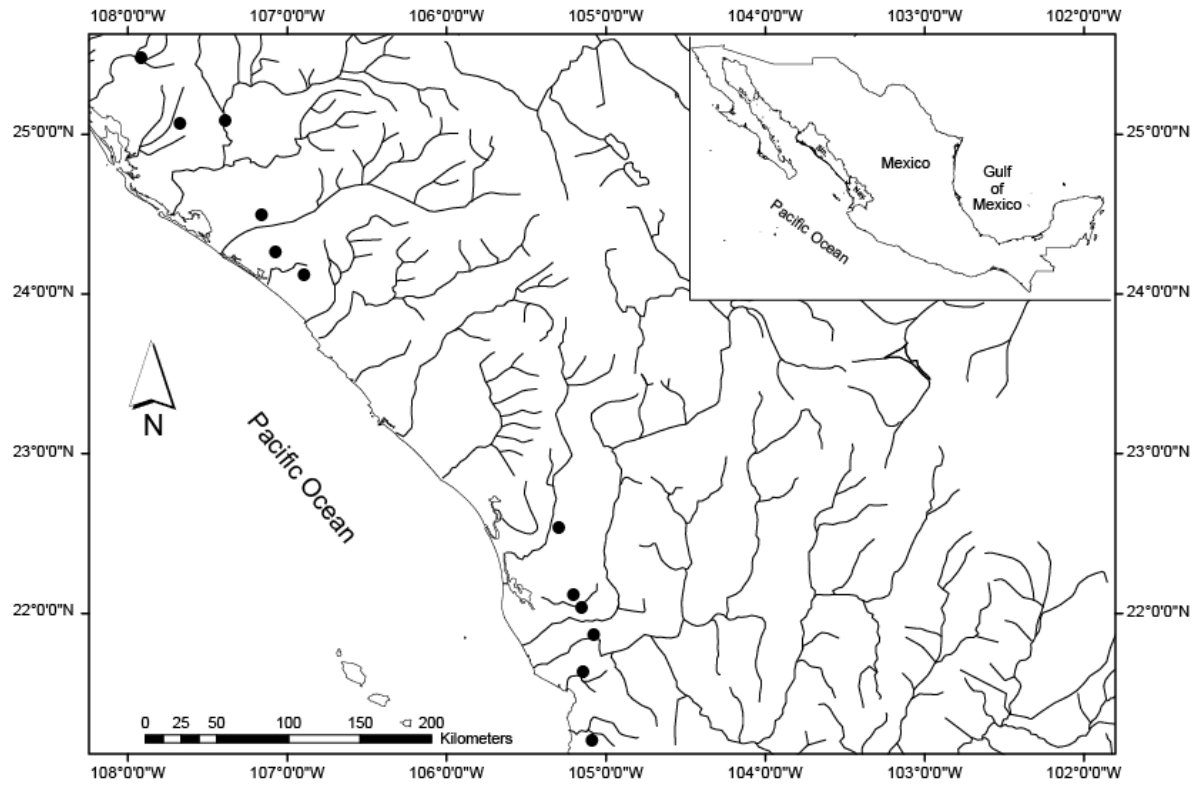
Figure 3.1: Map of locations sampled for *Poeciliopsis prolifica*. Solid dots indicate location sampled.
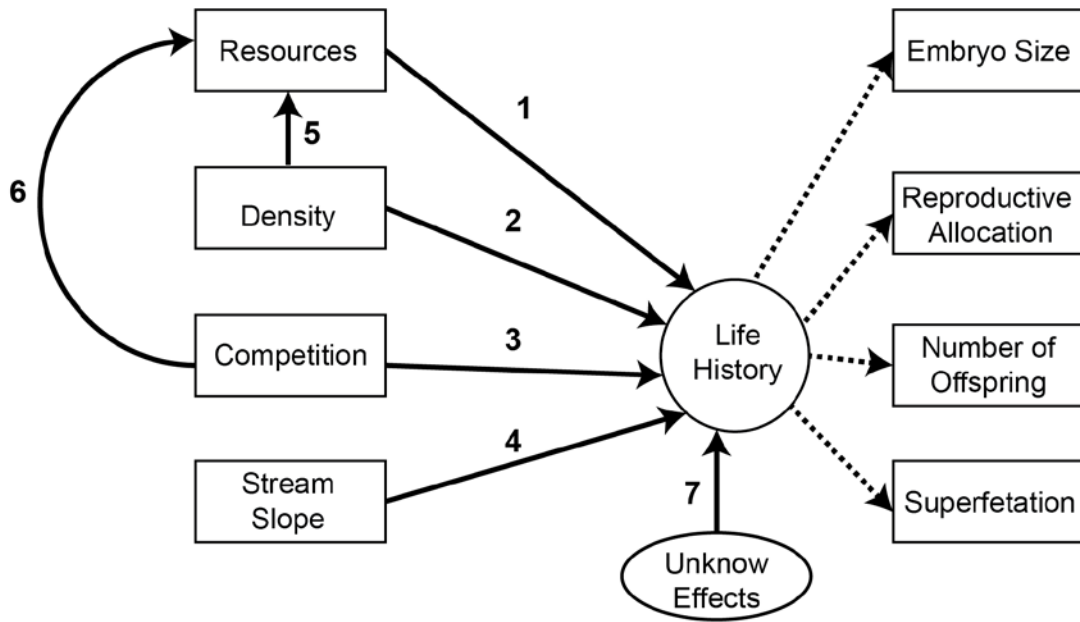
Figure 3.2: Global model path diagram of both direct and indirect effects of four putative selective agents on life history.
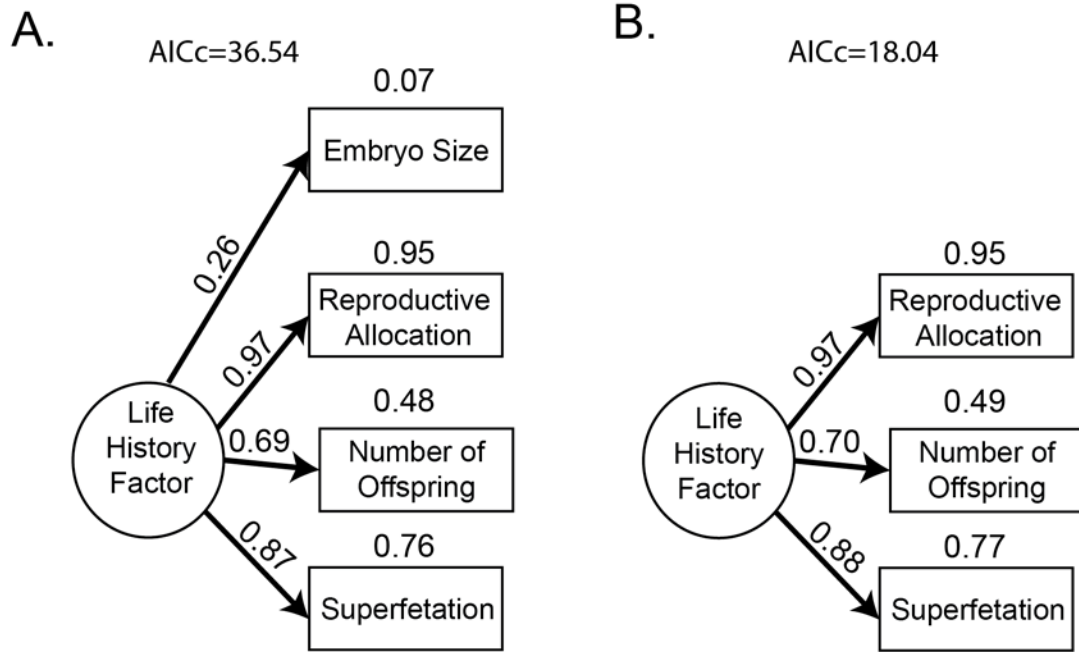
Figure 3.3: Path diagrams of confirmatory factor analysis to define the best representation of all four life history traits. A. Confirmatory factor analysis for all life history traits as a single measure, B. Confirmatory factor analysis excluding embryo size. Path diagram show loadings above the path and proportion of variance accounted for (R^2) above each life history trait.
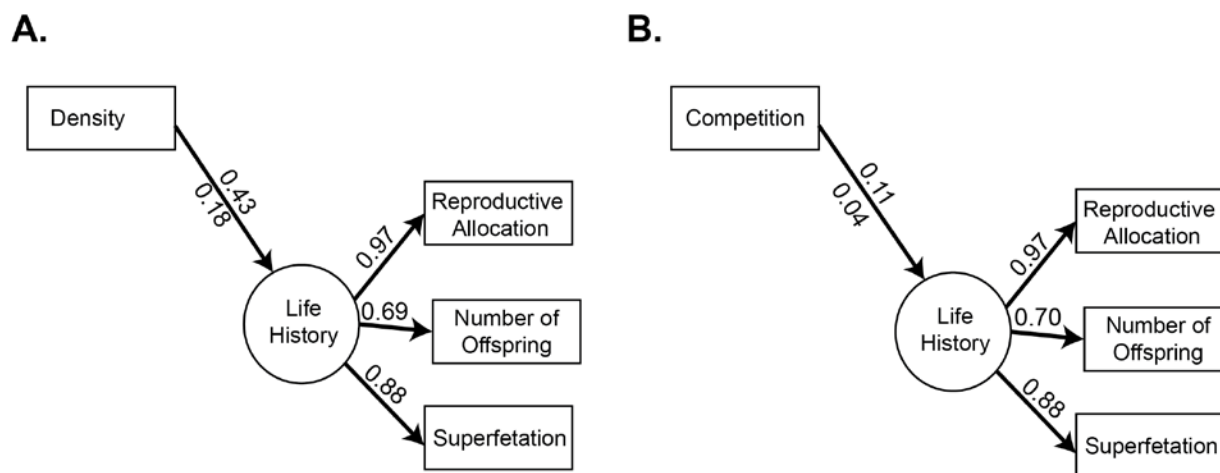
Figure 3.4: Path diagrams of best models as indicated by model selection on the life history factor. A. Path diagram of density as a direct effect on life histories, B. Path diagram of competition as direct effect on life histories. Path diagram shows total standard effects (above the path) and model-averaged total standard effects (below the path).
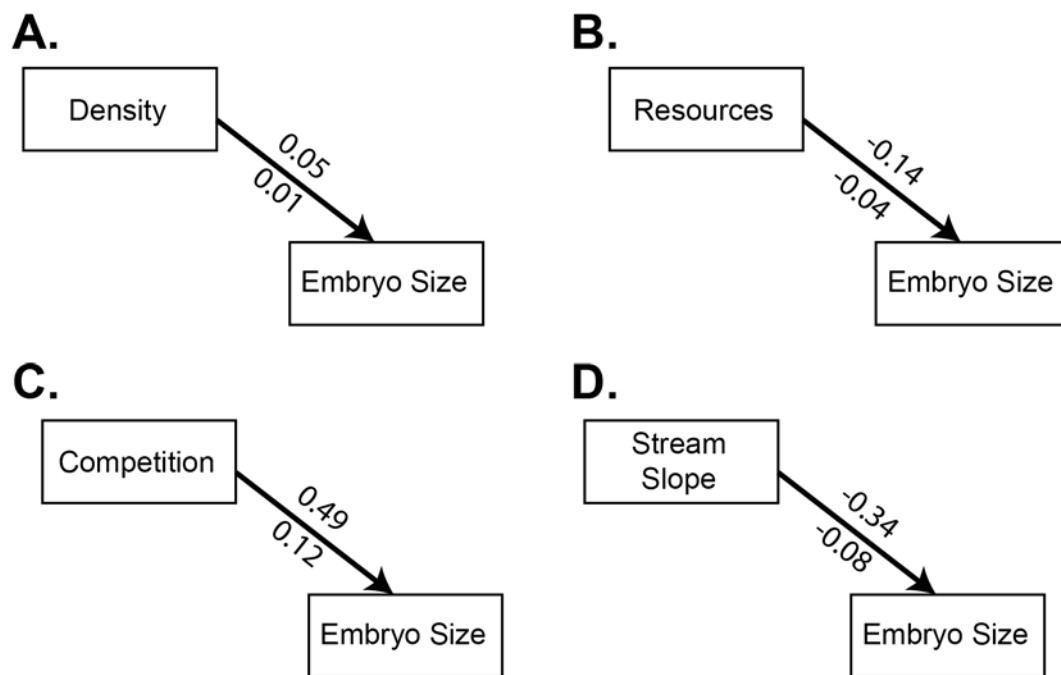
Figure 3.5: Path diagrams of best models as indicated by model selection on embryo size. A. Path diagram of density as a direct effect on embryo size, B. path diagram of resource availability as direct effect on embryo size, C. path diagram of competition as direct effect on embryo size, D. Path diagram of stream slope as direct effect on embryo size. Path diagram shows total standard effects (above the path) and model-averaged total standard effects (below the path).