

Cruise Speck

Plasticity and adaptive evolution in the body size and shape of Trinidadian guppies

Master's thesis in Biology
Supervisor: Cameron Ghalambor
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Abstract

Phenotypic plasticity describes predictable changes in a phenotype for a given genotype in response to different environments. Plasticity can affect evolutionary trajectories by moving populations either closer to (adaptive plasticity) or further from (non-adaptive plasticity) a locally adapted phenotype, thus affecting the strength of selection. This study investigates how plasticity in body shape and size influences evolution over a short timeframe. To test this, Trinidadian guppies were translocated from a high predation stream to four low predation streams and their body shape and size were measured over one year to quantify the immediate plastic response. Guppies from these populations, as well as three native low predation populations, were reared in a common garden to quantify evolutionary changes in shape and size. I find that size exhibited adaptive plasticity in the translocated guppies, shifting closer to that of the native low predation populations, while changes in shape were largely non-adaptive, shifting further away. In the common garden, little difference in size was seen between populations, suggesting adaptive plasticity shielded the trait from evolutionary divergence. Meanwhile, significant changes in shape, both adaptive and non-adaptive, were seen, indicating rapid evolution in the field. The most adaptive shape changes were exhibited by populations that showed the most extreme non-adaptive plasticity in the field, suggesting plasticity increased the strength of selection. These results demonstrate that plasticity plays a significant role in the direction and speed of phenotypic evolution.

Introduction

Evolution of multivariate traits

Local adaptation involves the evolution of a higher fitness advantage for genotypes in their home environment compared to genotypes from an away environment in response to divergent selection pressures (Kawecki & Ebert, 2004; Williams, 1966). A major challenge in evolutionary ecology is to understand how such divergent selection acts on multivariate traits and, by extension, how integrated phenotypes diverge among populations. The prevailing conceptual framework to explain how multivariate phenotypes evolve is captured in the “evolution along the lines of least resistance” hypothesis which is based on how direct and indirect selection act on the additive genetic variance and covariance described in the G-matrix (Lande & Arnold, 1983; Schluter, 1996).

The G-matrix describes the genetic variance for a trait and the covariance between traits, and thus describes how traits in a population are genetically correlated with one another (Arnold et al., 2008). Genetic pleiotropy and linkage are thought to underlie genetic correlations and result in patterns of integration that can be studied in the context of the G-matrix (Merilä & Hendry, 2014). The structure of the G-matrix biases how traits respond to direct and indirect selection and evolve towards the fitness optimum (Arnold et al., 2008). It is predicted that evolution is biased towards the genetic line of least resistance (g_{\max}), where the genetic variation between traits is greatest (Schluter, 1996). This can constrain evolutionary responses when there is selection for trait values outside of this major axis but can also facilitate a rapid and straightforward evolution towards the optimum when the path of selection is parallel with the major axis (Merilä & Björklund, 2004; Schluter, 1996).

More recently, models of adaptive evolution have attempted to introduce how phenotypic plasticity can affect the trajectory and speed of evolution (Draghi & Whitlock, 2012; Gibert et al., 2019; Lande, 2009; Price et al., 2003; Sgrò & Hoffmann, 2004). Phenotypic plasticity describes predictable changes in a phenotype for a given genotype in response to environmental cues and is a pervasive phenomenon across numerous traits and organisms (Sommer, 2020). Plasticity has long been assumed to help populations adapt to and colonise new environments over very short timescales, but it can potentially also play an important role in evolution by altering the structure of the G-matrix (Gibert et al., 2019; Noble et al., 2019; Sakata et al., 2020; Sgrò & Hoffmann, 2004; Wood & Brodie 2015, 2016) and the direction and strength of selection (Ghalambor et al., 2007, 2015; Price et al., 2003).

Indeed, despite the recognition that environmental context will influence estimates of heritability and additive genetic variance and covariance, relatively few studies have explored the consequences of plasticity for the structure of the G-matrix (Sgrò & Hoffmann, 2004; Wood & Brodie, 2015). Phenotypic plasticity can alter the strength of selection by shifting the distribution of phenotypes either closer to or further away from the local phenotypic optimum (Gibert et al., 2019; Ghalambor et al., 2007, 2015; Price et al., 2003). For example, plasticity can weaken the strength of selection when it is adaptive (i.e. the phenotypic change confers a fitness advantage and brings a population closer to the fitness optimum) or increase the strength of selection when it is non-adaptive (i.e. when the phenotypic change is moved further away from the fitness optimum; Ghalambor et al., 2007; Price et al., 2003; Schlichting & Pigliucci, 1998). Therefore, the way in which the plastic response alters genetic variance and the strength of selection can either constrain or facilitate evolution toward a phenotypic optimum. Selection may also lead to the evolution of plasticity itself, if there is genetic variation in plasticity within a population (i.e. variation in genotype x environment interactions or GxE; Via & Lande, 1985). GxE describes how genotypes produce phenotypes across different environments; when there is variation in GxE, there can be selection for genotypes that produce a more adaptive or favourable plastic response in a certain environment. Collectively, there are several lines of evidence suggesting phenotypic plasticity can play a significant role in the local adaptation of a population. However, few empirical studies have investigated how plasticity in multivariate traits impacts the evolutionary response to selection.

Guppies and body shape – a good study system

A useful study system to understand how multivariate traits evolve in the context of plasticity are Trinidadian guppies (*Poecilia reticulata*). Due to their short generation time, phenotypic evolution in guppies can be observed over short periods of time (Reznick et al., 1997). Fish body shape is a multivariate trait of interest in a number of studies investigating evolution and plasticity (e.g. Arnett & Kinnison, 2017; Burns et al., 2009; Schluter, 1993; Williams et al., 2017). Body shape and size affects fishes' manoeuvrability, swimming speed, and escape behaviour (Videler, 1993; Webb, 1982, 1984). Given its functional importance in aquatic environments, fish body shape is highly correlated with individual fitness and can differ significantly amongst populations in response to divergent selection (Walker, 2010). Like many traits, body shape and size in guppies has been shown to be plastic in response to a

variety of environmental variables, including flow regime, diet, habitat use, reproductive behaviour, and predation level (Dzikowski et al., 2004; Handelsman et al., 2014; Langerhans et al., 2004; Pakkasmaa & Piironen, 2001; Torres-Dowdall et al., 2012; Wimberger, 1992).

In guppies, body shape and size show repeated patterns of local adaptation in the Northern Range Mountains of Trinidad where levels of predation differ between habitats (Alexander et al., 2006; Ghalambor et al., in prep.; Hendry et al., 2006). Here, larger downstream rivers are typically inhabited by a number of predatory fish, while the smaller headwater streams tend to harbour few predators (Reznick et al., 2001; Seghers, 1973). It would be expected that guppies in high predation habitats evolve body shapes that allow them to better escape predators, for example more streamlined and fusiform shapes that facilitate faster burst speeds for escape (Fu et al., 2013; Williams et al., 2017). Body size can also be an important factor in predator evasion by allowing the fish to outgrow a predator's gape (Nowlin et al., 2006). However, in some species, including guppies, high predation selects for smaller body sizes at maturity as it imposes high mortality rates and predators tend to target larger guppies (Liley & Seghers, 1975; Reznick & Endler, 1982; Torres-Dowdall et al., 2012). Several morphometric analyses of guppies have indeed identified significant differences in body shape between guppies from low and high predation populations (Alexander et al., 2006; Burns et al., 2009; Hendry et al., 2006). For example, guppies inhabiting high predation areas have been found to have upturned mouths and a more fusiform shape, while those in low predation areas have deeper bodies and more anteriorly oriented mouths (Alexander et al., 2006).

Study aims

Here, I aim to understand how plasticity in body shape and size in guppies impacts evolutionary change following an experimental translocation from high to low predation streams. To do this, guppies were translocated from a high predation stream to four low predation streams and changes in body shape and size over one year were measured in the field. The translocated guppies were compared with the ancestral high predation source population they were derived from, as well as three native low predation populations in the same drainage (presumably locally adapted to low predation conditions). After one year (approximately 3-4 generations), guppies were collected from the wild and reared in common garden conditions to elucidate the genetic basis of changes in shape and size observed in the

field. Given that previous studies have shown that guppies are capable of rapid evolution, I predicted that:

- 1) if plasticity is adaptive, translocated high predation guppies will develop a body size and shape more similar to native low predation populations, but these changes should be constrained by patterns of covariance among traits.
- 2) when reared in common garden conditions, traits exhibiting adaptive plasticity in translocated guppies will show little evolutionary change because they are under weak selection, while traits exhibiting non-adaptive plasticity will exhibit evidence for evolutionary divergence from the source population because they are under stronger selection.
- 3) patterns of trait correlations described in the variance-covariance matrix will bias any evolutionary divergence observed.

Prior studies have undertaken translocation experiments from high to low predation streams in guppies and found rapid evolution in age and size at maturity (Reznick & Bryga 1987, 1996; Reznick et al. 1990, 1997). However, studies have not investigated the initial phenotypic changes that occur immediately after translocation – that is, those driven by phenotypic plasticity. Monitoring changes in the body size and shape one year following translocation can further provide a window into understanding how the plastic response of this multivariate phenotype impacts evolutionary change.

Methods

Field population introduction experiments

To understand how plasticity and evolution influence divergence in body shape and size, the progeny of Trinidadian guppies (*P. reticulata*) was translocated from a high predation section of the Guanapo River (Saint George, Trinidad) into four headwater streams where guppies and major predators were absent. Both the upper and lower limits of the introduction streams were bounded by barrier waterfalls, meaning that the immigration of guppies downstream and the emigration of guppies upstream was prevented. The first set of introductions took place in March 2008, where the Lower Lalaja (LL) and Upper Lalaja (UL) streams were stocked with 38 gravid females and 38 mature males. A visible subcutaneous implant elastomer tag was placed on each guppy for identification purposes (NorthWest Marine Technology, Shaw Island, WA, USA). In March 2009, the experiment was replicated with a

second group of 45 gravid females and 45 mature males which were translocated to the Caigual (IC) and Taylor (IT) streams.

Each month following the initial translocations, all newly recruited individuals above 14mm in standard length were collected from each introduction stream and transported to a field laboratory, where they were marked with a unique elastomer tag and photographed for morphometric analysis. Lateral photographs of the left side of each guppy were taken with a Nikon D60 digital SLR camera using a Nikkor 50mm macro lens (Nikon Inc., Melville, NY, USA) mounted on a tripod. Tripod height was set to provide an 8cm field of view, which eliminated any parallax affecting the image of the guppy. A fine-tipped wetted artist's paintbrush was used to straighten the specimen and spread the fins to standardise fish position and expose homologous landmarks. The guppies were returned to their respective stream after being processed in the laboratory. Monthly sampling was conducted for 12 months; see Table 1 for sample sizes of each population for each month.

Sampling also occurred in the high predation source and existing low predation populations in order to have reference points of the ancestral and expected derived body size and shape. In March 2008, wild-caught adult males were sampled from the source population stream and from two native low predation populations in the Caigual (CL) and Taylor (TL) tributaries. The CL and TL sites were located downstream from the introduction populations and sampling occurred prior to the translocations, thus there was no opportunity for mixing of introduced and native individuals. In March 2012, wild-caught adults were also sampled from a third native low predation population in the Tumbason (TB) tributary in the Guanapo River. These individuals were measured and photographed in the laboratory as above. All three native low predation populations sampled were within the Guanapo drainage and are assumed to be derived from the same downstream source population as the introduction fish (e.g. Willing et al., 2010), however have been isolated for much longer periods of time.

Common garden experiment

After 12 months, juvenile male and female individuals were collected from each of the eight populations (four introduction populations, three native low predation populations, and source population) and brought to a laboratory. Here the fish were reared under identical conditions (i.e. controlled light levels, temperature, and feeding regimes) for two generations up to adulthood, in order to control for maternal and environmental effects. Male and female

offspring of wild-caught individuals were kept separate to create unique family lines. Upon maturity, unique crosses were made between family lines to maintain genetic variation within each population and these second-generation offspring were measured for body shape under identical common garden conditions. Specifically, fish were held in 1.5L recirculating tanks (Aquatic Habitats, Apopka, FL, USA) and maintained on a 12-hr light cycle at 27°C. Food quantity was based on the high food level administered in Reznick (1982), adjusted for the age and number of individuals in the tank (Tetramin[®] tropical fish flakes, Spectrum Brands, Inc., Cincinnati, Ohio, USA for morning feeding; brine shrimp nauplii (*Artemia*) for nightly feeding).

The second-generation fish were divided into recirculating tanks and randomly assigned to either a ‘predator cue’ or ‘no predator cue’ treatment. In the predator cue treatment, the water circulated through a tank that contained a pike cichlid (*Crenicichla lenticulate*) that was fed guppies. The guppies in this treatment would therefore be exposed to predator kairomones and conspecific alarm cues. Normal untreated water was used in no predator cue treatment (see also Ghalambor et al., 2015; Handelsman et al., 2013). See Table 2 for sample sizes of each population in the treatments. Comparisons between full siblings in the predator and no predator cue treatments allow for testing of the magnitude of plasticity, while comparisons between the introduction populations and the source population allow for testing of evolutionary divergence. When evolutionary changes in the introduction populations mirror the patterns observed in native low predation populations, it is assumed these changes reflect adaptive evolution.

Shape capture and landmarking

The lateral body shape of the guppies was quantified using a geometric morphometrics approach (Rohlf & Marcus 1993; Zelditch et al. 2004). The photographs taken of each guppy in the field and laboratory were loaded into TPSDig2 to be landmarked. Eight fixed landmarks and six sliding semi-landmarks were placed to capture the lateral morphology of each individual (Fig. 1; Bookstein 1997). Fixed landmarks are those placed at anatomically homologous locations, while semi-landmarks consist of a set of landmarks that are not strictly homologous (Fig. 1).

Statistical analyses

The shape data were analysed in R using the *geomorph* package (Adams & Otárola-Castillo, 2013). A generalised Procrustes analysis was performed to eliminate variation in shape due to differences in the size, orientation, and position of the images (Goodall, 1991; Rohlf & Slice, 1990). These Procrustes-aligned shape data were then used in all subsequent statistical analyses. A principal component analysis (PCA) was performed (separately for field and laboratory specimens) to identify and plot the axes of greatest shape variation amongst individuals. The *plotRefToTarget* function was used to visualise differences in body shape represented at the minimum and maximum values of the PC axes (see Figs. 4, 5, 8, 9). Previous research has shown that the patterns of phenotypic correlations measured under common garden conditions provide “fair estimates” of the underlying genetic correlations (Cheverud, 1988; Roff, 1995), thus the phenotypic P-matrix (represented by the relationship between the first two principal components) is used here a proxy for the G-matrix when examining the multivariate changes in body shape.

A Procrustes ANOVA was run separately for field and laboratory specimens. A Procrustes ANOVA calculates the Procrustes distance among specimens and quantifies the relative amount of variation in Procrustes distance to the predictors in the model. P-values are calculated by estimating the probability of the variation observed compared to distributions randomly generated from resampling permutations (here set at 10,000). The field model included the shape data as a response variable, with centroid size as a covariate and population as a predictor variable (see Table 3). Centroid size is an estimate of the average distance between each landmark and the centroid (the mean coordinates of the specimen) and is used as a proxy for body size. The interaction between centroid size and population (centroid:population) was also tested for in the model. Only individuals sampled at month 12 from the introduction populations were included in the model. A post-hoc pairwise test was run on this model to identify which populations differed significantly in shape (see Table 4). The null model in the pairwise analysis was defined with only centroid size as a predictor variable (i.e. shape ~ centroid), as this is the simplest model that accounts for allometric effects of size on shape. Due to a significant centroid:population interaction found in the model, another post-hoc pairwise test was run with the null model defined as the original model without the interaction (i.e. shape ~ centroid + population). This was to identify which populations differed significantly in their allometric slope (see Table 5). The laboratory model was identical to the field model, except that treatment (predator cue/no predator cue)

was also included as a predictor variable. Interactions between all predictor variables were included – centroid:population, centroid:treatment, population:treatment, and centroid:population:treatment (see Table 6). A post-hoc pairwise test was then run to identify which population-treatment combinations differed significantly in shape (see Table 7).

Results

Body shape and size changes in the field

Body size (centroid size) varied between populations in the field; the source population had the lowest average body size, while the native low predation populations had the highest (21% larger than the source population; Fig. 2). After one month, the translocated guppies had a much larger body size, closer to that of the native low predation populations. Average body size in the introduction populations fluctuated slightly over 12 months, however remained relatively consistent. After 12 months, guppies from the four introduction populations combined were on average 16% larger than the source population. Moreover, a significant relationship between body size and body shape ($F = 77.42$, $P < 0.0001$) was found, as well as a significant interaction between body size and population ($F = 1.56$, $P = 0.0165$). This indicates the presence of allometry, and differences in allometric trends between the sampled populations. All populations but the source and TL population exhibited a negative allometric relationship along PC1, where larger individuals had lower PC1 values (shorter caudal peduncles and narrower heads; Fig. 3). Pairwise analyses revealed that the LL population differed significantly in its allometric slope from all other native and introduction populations (Table 5). Specifically, LL had a significantly steeper negative slope than the other populations (Fig. 3). A significant difference was also found between the source and IT populations; similar but non-significant differences were found between the source and IC populations (Table 5).

The Procrustes ANOVA with body size as a covariate revealed significant differences in body shape between the field-sampled populations ($F = 45.49$, $P < 0.0001$). No significant differences in shape were found between the LL and UL populations, nor between the IC and IT populations; however, LL and UL differed significantly from IC and IT. The source population differed significantly from the three native low predation populations, as well as from the four introduction populations. See Table 4 for pairwise P-values. The first two principal component axes accounted for 58.44% of variation in body shape in the field-

sampled guppies. PC1 explained 39.53% of shape variation and was characterised by lengthening and upturning of the caudal peduncle and shortening and narrowing of the head (Fig. 4). PC2 explained 18.91% of shape variation and was characterised by upturning of the mouth and caudal peduncle (Fig. 5). The relationship between PC1 and PC2 (an approximation of the P-matrix) was similar between the source and introduction populations (Fig. 6a,b). However, variance in shape was much higher in the LL and UL populations than the source population (Fig. 6a). Variance was similar to the source population in the IC and IT populations (Fig. 6b). More variation in the P-matrix was seen between the source and native low predation populations (Fig. 6c). Notably, the TB population exhibited a stronger correlation between PC1 and PC2, while a much weaker correlation was exhibited by the CL population (Fig. 6c).

The four introduction populations were all characterised by high PC1 scores relative to the source and native low predation populations (Fig. 4). Of these, the IC and IT populations had the highest PC1 scores. The change in shape characterised by PC1 occurred within one month of translocation, with shape fluctuating over time but remaining similar between the initial (month 1) and final (month 12) measurements (Fig. 4). However, the IC and IT populations differed greatly from the LL and UL populations along PC2 (Fig. 5). LL and UL shared similar scores to that of the source and native low predation populations, while IC and IT had significantly higher scores by the end of the experiment. The divergence in shape characterised by PC2 in the IC and IT populations did not occur until after six months following translocation (Fig. 5). There is therefore a particularly notable shape divergence in IC and IT where the caudal peduncle lengthened, the head shortened, and the mouth upturned to a degree beyond that which is seen in the low predation native populations. The upturning of the mouth, as characterised by high PC2 scores, was not seen in any of the other (native or introduction) populations.

Body shape and size under common garden conditions

The large differences in body size between populations in the field were not seen under common garden conditions. Of the introduction populations, LL and UL had the largest average body sizes, while IC and IT had the lowest (Fig. 7). Body size for the source population was intermediate – lower than that of the LL and UL populations, but higher than that of the IC and IT populations. Interestingly, body size differed markedly between the three native low predation populations – TB had the lowest of all populations (both native

and introduction), while TL had the highest. For all populations, individuals reared without a predator cue were on average larger than those reared with a predator cue. When reared with a predator cue, the differentiation between the populations changed significantly – body size in the UL and IC populations decreased by 10% and 7% respectively and converged, while the LL population had the highest body size of all populations. While there was a significant relationship between body size and body shape ($F = 24.96$, $P < 0.0001$) amongst laboratory guppies, there were no significant interactions between body size and population ($F = 1.04$, $P = 0.3830$) or body size and treatment ($F = 0.92$, $P = 0.4670$), meaning allometric trends were consistent between populations and treatments, unlike in the field.

With body size accounted for, significant differences in body shape between populations in the laboratory were found ($F = 18.96$, $P < 0.0001$). The first two principal component axes accounted for 54.89% of variation in body shape in the laboratory-sampled guppies. PC1 explained 35.76% of variation and was characterised by deepening of the body, shortening of the caudal peduncle, and elongation of the head (Fig. 8). PC2 explained 19.13% of variation and was characterised by downturning of the caudal peduncle, narrowing of the abdomen, elongation of the head, and downturning of the mouth (Fig. 9). In the common garden, larger differences in the P-matrix between the source and introduction populations were found compared to the field. Under the no predator cue treatment, the relationship between PC1 and PC2 in the IC population was opposite to that of the source population (Fig. 10b). Specifically, in the IC population, the major axis of variation was parallel with PC2, while in the source population the major axis was parallel with PC1. In contrast, the native low predation populations had more similar P-matrices to the source (Fig. 10c, 11c). Some introduction populations also exhibited less variation in shape than the source, most notably IC in the no predator cue treatment (Fig. 10b) and IT in the predator cue treatment (Fig. 11b).

Reared without a predator cue, the native low predation populations had the highest average PC1 scores, while the source population had lowest (Fig. 8). While not showing the degree of divergence seen in the field, the introduction populations had higher PC1 scores than the source (Fig. 8), suggesting a genetic basis for the phenotypic divergence. However, pairwise analyses revealed that the IC population did not differ significantly in shape from the source population ($P = 0.3833$; Table 7). The IT and LL populations were closest to the native low predation populations on PC1 (Fig. 8). The IC and IT populations diverged significantly from the LL and UL populations in shape characterised by PC2 (Fig. 9). LL and UL had the lowest PC2 scores, while IC and IT had the highest PC2 scores, closer to the

native low predation populations (Fig. 9). This suggests a difference in the genetic divergence between these two sets of populations. The source population placed intermediately along PC2, scoring significantly higher than LL and UL, but lower than IC and IT. This indicates the development of a more upturned mouth and deeper abdomen in LL and UL and the opposite in IC and IT (closer to the native low predation morphospace), compared to a more intermediate morphology in the source population.

A significant difference in shape between the predator cue and no predator cue treatments was found ($F = 9.63$, $P < 0.0001$). Furthermore, the interaction between population and treatment was significant ($F = 1.63$, $P = 0.0085$), meaning the difference in shape between treatments varied between populations. In all populations, individuals reared without a predator cue had higher PC1 and lower PC2 scores than those reared with a predator cue (Figs. 8, 9). However, these differences in shape between treatments were only significant in the introduction populations, namely LL ($P = 0.0481$) and UL ($P = 0.0338$; Table 7). Similar but non-significant differences between treatments were found in IC ($P = 0.0676$) and IT ($P = 0.2713$; Table 7). When exposed to a predator cue, the introduction populations moved closer to the source along PC1 (with the exception of IC; Fig. 8). Along PC2, LL and particularly UL saw the largest increase in score, moving them closer to the source; the increase in IC and IT moved them further away from the source and closer to the native low predation populations (Fig. 9). This suggests that the LL and UL populations experienced plastic morphological changes between different treatments, where the presence of a predator cue triggered a narrowing of the body, downturning and lengthening of the caudal peduncle, and downturning of the mouth.

Discussion

The relationship between plasticity and adaptive evolution is complex as the environmental context can impact patterns of trait correlations, the amount of heritable variation expressed, and the strength of selection (Sgrò & Hoffmann, 2004; Wood & Brodie, 2015). Such context-dependency requires empirical studies that capture the role of plasticity during the early stages of adaptive evolutionary divergence between populations in order to understand how traits evolve. Here I find that guppies translocated from a high to a low predation environment exhibit adaptive plasticity in body size (Fig. 2) and some aspects of body shape (Fig. 4), while other aspects of shape appear non-adaptive (Figs. 4, 5). Under common garden

conditions I find evidence for rapid evolutionary divergence, with some changes in a clearly adaptive direction, but others in non-adaptive directions (Figs. 8, 9). Below I discuss how these patterns can be interpreted in the context of genetic and phenotypic correlations and how plasticity impacts the strength of selection.

Adaptive plasticity in body size constrains evolution

The changes in body size observed in the introduction populations suggest an adaptive plastic response (Fig. 2). Body size increased significantly in the introduction populations in the first few months, becoming much closer to (but not exceeding) that of the native low predation populations. Therefore, the introduction populations were brought closer to the assumed new phenotypic optimum, presumably reducing the strength of selection on these populations. Larger body sizes at maturity are typical of low predation populations (Magurran, 2005; Reznick, 1982; Reznick & Endler, 1982) and prior translocation studies have also shown that low predation guppies develop larger bodies (Reznick et al., 1997). However, the patterns of body size in the field, where the introduction and native low predation guppies were significantly larger than the high predation source guppies, were not found under common garden conditions (Fig. 7). While body size in the source population was similar in both the field and laboratory environments, the native and introduction low predation populations matured at smaller sizes than their field-raised counterparts, resulting in little difference in body size from the source. Like in the field, body size showed plasticity in the laboratory. When reared with a predator cue, body size in all populations was lower than when reared without a predator cue (Fig. 7). This plastic response aligns with that in the field, where body size increased in the low predation introduction populations. Moreover, these differences between treatments are consistent with previous research which has found similar effects of predation on body size in guppies (Reznick, 1982; Reznick & Bryga, 1987, 1996; Reznick et al., 1990, 1997). While the LL and UL populations shared a similar body size with the source, the IC and IT populations shared a relatively lower body size with the native low predation populations (Fig. 7). Reznick et al. (2019) found that increased population density was the main driver of selection of larger sizes at maturity in guppies, thus the differences in body size seen between the introduction streams could be due to density-related variation. The larger body sizes in LL and UL may could therefore indicate that these populations experienced higher population densities (possibly due to environmental factors such as increased food availability).

These results suggest that the differences in body size between high and low predation guppies seen in the field are not the result of genetic divergence, but rather plasticity. Since larger body sizes typically evolve in low predation habitats in guppies, the plastic response observed here appears to be adaptive. The significant adaptive plasticity acting on body size in these populations is likely shielding this trait from selection, resulting in convergence towards a common body size when environmental differences are controlled. This plastic response may have evolved to allow body size in these guppies to be highly flexible, where it can change with changing predator conditions (or other environmental conditions) in order to maintain high fitness. Studies in other fishes have similarly found plasticity in body size (Burns et al., 2009; Januskiewicz & Robinson, 2007; Preisser & Orrock, 2012), however a study in mosquitofishes found plasticity in body shape but not body size in response to predator cues (Arnett & Kinnison, 2017).

Non-adaptive plasticity in body shape facilitates evolution

Numerous studies have found that body shape in fish is highly plastic in response to various environmental factors (Robinson & Parsons, 2002), including predation (Arnett & Kinnison, 2017), and our results here suggest no different. Guppies in all four introduced populations diverged significantly from the ancestral population in size and shape within a month, suggesting a rapid plastic response in the phenotype. Specifically, the introduced guppies exhibited a larger caudal peduncle and a narrowing of the head and anterior section of the body (higher PC1 scores; Fig. 4). Other studies in fish have similarly found a plastic shift towards a larger caudal peduncle and a narrower body in the presence of predators (Arnett & Kinnison, 2017), which aligns with the morphologies seen in fish that permanently inhabit low predation streams (Alexander & Breden, 2004; Langerhans & Dewitt, 2004). This plasticity-driven shift away from the source population in the direction of the native low predation populations' morphologies following translocation suggests a degree of adaptive plasticity. However, these body shape changes actually shifted the introduced populations further away from the assumed optimum occupied by the native low predation populations and, as such, may be non-adaptive in the new environment.

Some aspects of shape in the introduced populations diverged in more unique ways not observed in the native low predation populations. Namely, guppies from the IC and IT populations had a significantly more upturned mouth and caudal peduncle by the end of the translocation experiment – a morphology atypical of low predation guppies (higher PC2

scores; Fig. 5). The development of this distinct morphology did not begin occurring until after six months following introduction, which could suggest a delayed plastic response or the beginning of an evolutionary response in these populations. This change may also have been the result of some unmeasured environmental change that occurred at this time. The development of these extreme and novel shape patterns in the IC and IT populations suggests that these components of shape might be under stronger selection because they are further away from the optimum (Gibert et al., 2019; Ghalambor et al., 2007; Price et al., 2003).

With each population reared in controlled laboratory environmental conditions, the phenotypic differences between the introduction and source populations provide evidence for genetic divergence and thus rapid evolution occurring in the field (over 3-4 generations). In the common garden, the native low predation populations were clearly differentiated from both the source and introduction populations on PC1 in both treatments, having deeper bodies and shorter caudal peduncles (higher PC1 scores; Fig. 8). Smaller but significant differences in shape were seen between the source and introduction populations when reared without a cue, showing evidence of genetic divergence (Fig. 8). Along PC2, the LL and UL populations diverged considerably from the IC and IT populations; both sets of populations differed from the source along PC2, but in opposite directions (Fig. 9). Specifically, LL and UL guppies developed a more upturned mouth and caudal peduncle and a deeper abdomen, a morphology similar to the novel phenotype the IC and IT populations developed through plasticity in the field (Fig. 5). Evolution of a deeper body in fish in low predation habitats has been documented previously (Williams et al., 2017), however in guppies only when paired with an anteriorly oriented mouth (Alexander et al., 2006). An upturned mouth is typically considered an adaptation to high predation environments, suggesting there may be underlying genetic correlations in these populations where mouth orientation evolves in tandem with other traits under heavy selection, like body depth (see next section; Handelsman et al., 2014).

Given only 3-4 generations of evolution, it is expected that a smaller amount of divergence than that which is driven by phenotypic plasticity in the field will be seen. Furthermore, the plasticity in shape occurring in the field could be affecting the evolutionary trajectory towards a locally adapted body shape. Indeed, the IC and IT populations, which exhibited the greatest morphological divergence from the low predation native populations in the field due to the development of an upturned mouth (higher PC2 scores, Fig. 5), had a more similar mouth orientation to the native low predation populations in the laboratory under both treatments (compared to LL and UL; Fig. 9). Along PC1, the IT population was

also significantly closer to the native low predation populations than the other introductions, however the IC population was interestingly the least similar (Fig. 8). These findings suggest that evolution could be accelerated by the presence of countergradient variation in the introduction populations, whereby the environment induces phenotypic changes that are opposite to those favoured by natural selection (Conover & Schultz, 1995). In line with this, Ghalambor et al. (2015) found that gene expression patterns in LL and UL evolutionarily diverged from the source population toward a native low predation population, and most of this divergence was only seen in transcripts that exhibited non-adaptive plasticity. This supports the hypothesis that non-adaptive plasticity and countergradient variation facilitates evolution by increasing the strength of directional selection, as suggested by existing models and frameworks (Paenke et al., 2007; Price et al., 2003).

Plasticity in body shape in response to a predator cue was observed only in two of the introduction populations (LL and UL). The IC and IT populations showed similar but non-significant differences between treatments. The lack of plasticity in the source or native low predation populations suggests that plasticity in body shape in response to predation evolved rapidly in the field. When reared with a predator cue, the introduction populations tended toward a phenotype more similar to the source (Figs. 8, 9). The IT and UL populations did not differ significantly from the source under the predator cue treatment, while they did under the no predator cue treatment which mimics the low predation environment (Table 6). Interestingly, the IC population differed significantly from the source only under the predator cue treatment, where a more extreme version of the ancestral phenotype was developed (Table 6). This suggests there is an effect of plasticity where the introduction populations move back towards the ancestral phenotype when they return to a predator-present environment. Torres-Dowdal et al. (2012) found that guppies from low predation localities exhibited a stouter head shape and more anteriorly positioned mouth when reared without versus with predator cues, while those from high predation localities did not change across treatments. This supports our findings here that plasticity has evolved from a non-plastic ancestral genotype, however leaves the question as to why a similar response was not seen in the native low predation populations.

In addition to the clear plasticity seen in shape and size in the field, the results suggest that the relationship between size and shape (i.e. allometry) may also be plastic. The LL population exhibited an allometric trend significantly different from the source population (as well as the three other introduction populations), and the IC population also differed from the

source population (Fig. 3). Plastic relationships between size and shape have been found in other studies, for example in threespine sticklebacks where the shape changes that occurred as fish grew with age were different when reared in complex versus simple habitats (Wund et al., 2012), and in *Daphnia*, where predator-induced allometric changes have been observed (Gu et al., 2021). Given that these allometric differences were not seen in the common garden under different predator treatments, and that it is only seen in some of the introduction populations, this plasticity may not be predator-induced. In the LL population, smaller individuals had body shapes much closer to that of the native low predation populations, while larger individuals exhibited the more extreme shape seen in the introduction populations (Fig. 3). This suggests an interesting effect of plasticity on the phenotype, where, presumably, fitness is simultaneously decreased in the low predation habitat due to a smaller body size and increased due to a more adaptive body shape. Previous studies have suggested that it is difficult for allometric slopes to evolve over time and as such could act as a constraint in body shape evolution (Bookmythe et al., 2016; Egset et al., 2012). This idea is supported by the lack of differences in allometry between populations in the common garden seen here, and similar results have been found in previous studies (Broder et al., 2020). If plasticity is able to more easily shift allometric slopes, as suggested here, it could help release populations from allometric constraints and influence evolutionary trajectories towards the optimum. The LL population did indeed exhibit the largest variance in shape of the introduction populations (Fig. 6a,b), which could suggest a release from correlative constraints.

The role of the G-matrix

The non-adaptive changes in shape observed in the introduction populations may suggest there is a misalignment of the direction of plastic changes with the major axis of genetic covariation (g_{\max}). Draghi & Whitlock (2012) hypothesised that plasticity and g_{\max} should be aligned, however this assumes a stable G-matrix. While here genetic correlations between morphological traits have not been measured in the wild populations, the shape of the P-matrix (as represented by the correlation between the first two principal components) can be observed to understand how stable or plastic trait correlations are between the different populations. Indeed, the results suggest that divergence in shape in the introduction populations did not occur along the major axis of variation (Fig. 6a,b). While the direction of the major axis remained similar between the source and introduction populations, there was

much more variance in shape in the LL and UL populations in comparison to the source population (Fig. 6a). Interestingly however, similar differences in variance in the IC and IT populations, where non-adaptive shape changes were seen most, were not seen (Fig. 6b). The shape of the P-matrix and direction of the major axis was much more different between the source and native low predation populations, and, in addition, variable between the native low predation populations (Fig. 6c). This suggests that trait correlations are not stable over evolutionary time and have evolved in response in low predation conditions (and likely other environmental variables). The comparison of the introduction populations and the native low predation populations also suggests that there is a release from the developmental and genetic constraints during the early stages of divergence that loosens shape trait correlations in these populations and provides more variation for selection to act upon.

Under common garden conditions where all individuals experience similar environmental conditions, the P-matrix provides a good estimate for the G-matrix and can provide insights into genetic constraints (Cheverud, 1988; Roff, 1995 – referred to as Cheverud’s conjecture). In the common garden, larger differences in the P-matrix were found between the source and introduction populations than in the field (Fig. 10a,b). In contrast, the native low predation populations had more similar P-matrices to the source population (Figs. 10c, 11c). Notably, compared the source, the relationship between PC1 and PC2 flipped entirely in the IC population when reared without a predator cue (Fig. 10b). This suggests that shape trait correlations are highly evolvable and likely take non-linear trajectories towards local adaptation. The flexible nature of these correlations in both the field and common garden could indicate that the G-matrix is not a significant constraint on shape evolution in these populations. Indeed, several studies suggest that the structure of the G-matrix is in many cases labile in response to environmental change (Sgrò & Hoffmann, 2004; Wood & Brodie, 2015). Further studies that measure the genetic correlations between morphological traits and how they change between these populations would provide further clarity into its role as an evolutionary constraint. Beyond the G-matrix, there may be various environmental variables that differ between the source and introduction streams that have affected the plastic response and contributed toward the development of a novel, non-adaptive shape (see next section).

Other potential drivers of body shape and size

Clearly less extreme plastic changes were seen in the common garden in comparison to the field. Furthermore, the plastic changes that occurred in the field were notably different from those in the common garden. For example, the phenotype of an upturned mouth that developed in the field in the IC and IT population was not observed in these same populations in the laboratory, but rather in the LL and UL populations. Differences between laboratory and field could be attributed to other environmental factors that differ between high and low predation streams in the field. While in the common garden the effect of predator presence alone is isolated and tested, low and high predation habitats can differ in structural complexity, food availability, canopy openness, and density. For example, guppies from high and low predation areas have been shown to differ in food preference (Bassar et al., 2010; Zandonà et al., 2011). Furthermore, body depth has been shown to be associated with structural complexity and benthic foraging behaviour in sticklebacks (Schluter & McPhail, 1992) and perch (Svanbäck & Eklöv, 2002). Therefore, plasticity and selection occurring in the field could be in response to differences in factors like foraging behaviour, and not purely the presence or absence of predators. Canopy openness did differ between introduction streams – the LL and IC populations had closed canopies while the canopies of the UL and IT populations were thinned – and has been found to be a highly important factor in determining guppy body size (Hendry et al. 2006). Open canopies have higher productivity which increases growth rate, and thus can increase competition and density, leading to larger guppies (Grether et al., 2001; Reznick et al., 2001, 2019). Guppies from the thinned canopy streams (UL and IT) did have the highest body sizes in the field of the four introduction populations at month 12 (Fig. 2). However, body sizes were not highest in the thinned canopy streams in the common garden (Fig. 7). This could suggest that canopy openness had an effect on the plastic response in body size in the field but did not lead to any genetic divergence within a year.

Different areas of the body are also likely under stronger selection from these various pressures. For example, head shape can be linked to foraging behaviour (benthic versus surface feeding; Robinson & Wilson, 1995). Surface feeding is more common in high predation localities due to the ability of guppies to jump out of the water and avoid predators. Moreover, guppies in high predation streams feed primarily on invertebrates, while low predation guppies have a more generalist diet consisting of invertebrates, algae, and detritus (Dussault & Kramer, 1981; Zandonà et al., 2011), which can cause divergent selection on

head shape. Thus, interactions between predation risk and diet can explain why high predation guppies in nature tend to have upturned mouths. The differences in head shape seen between guppies from low and high predation streams align with those seen between sticklebacks from limnetic and benthic environments, where the differences in diet and foraging behaviour are similar (Schluter & McPhail, 1992). In contrast, caudal peduncle shape is likely more driven by flow rate or predator presence due to its role in swimming performance and predator escape (Langerhans & Reznick, 2009; Webb, 1978). Therefore, unexpected patterns such as the mouth orientation shift in the IC and IT populations seen in the field might be in response to other environmental factors like foraging behaviour or food availability, especially considering this change did not occur until mid-way through the experiment (where there might have been a sudden change in the environment, like in food availability).

Conclusions

Our results clearly demonstrate a complex interplay between plasticity and evolution in the adaptation of body shape and size in Trinidadian guppies. Both adaptive and non-adaptive plasticity were observed in the field and common garden. Notably, body size was clearly plastic in an adaptive direction in guppies translocated from high to low predation streams. This appeared to shield this trait from selection, as suggested by the lack of differences in body size between populations under common garden conditions. Plasticity in body shape was, in contrast, largely non-adaptive. While some aspects of shape moved in an adaptive direction, the extent of the change moved the introduction populations further away from the body shapes exhibited by native low predation populations. The populations where the most novel body shape changes occurred in the field (IC and IT populations) exhibited more adaptive evolutionary change in the common garden, suggesting that non-adaptive plasticity increased the strength of selection. While actual genetic correlations in these populations were not measured here, the flexible nature of correlations between the major aspects of body shape in both the field and common garden could suggest that genetic correlations do not act as a significant constraint on body shape evolution and instead change with the environment. This is contrary to my prediction that plastic changes would be constrained by patterns of covariance between traits.

Translocation experiments that track body size and shape changes for a period beyond one year could provide more information on the phenotypic trajectory of these populations.

Measuring other environmental variables such as flow rate and density in each stream could also provide insight into the role of other factors beyond predation level on shape and size. Furthermore, actual measurements of genetic correlations in these populations would allow us to better understand the role of the G-matrix in evolution, as well as its relationship with plasticity. While our results suggest the potential for plasticity to release populations from allometric constraints, more studies that compare evolutionary and plastic changes in allometry over longer periods of time and its effect on shape would be useful in understanding its role in shape evolution.

Tables and Figures

Tables

Table 1: Sample sizes of each population in the field translocation experiment.

Sample sizes for native field populations	
Source	67
Caigual LP	21
Taylor LP	33
Tumbason LP	19

Monthly sample sizes for introduction populations												
	1	2	3	4	5	6	7	8	9	10	11	12
Lower Lalaja Intro	5	5	26	49	41	42	39	57	55	68	58	56
Upper Lalaja Intro	8	9	44	52	24	20	22	35	33	36	41	57
Caigual Intro	52	37	34	29	31	44	47	43	48	54	52	57

Taylor Intro	45	25	48	76	62	24	19	21	32	40	46	56
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Table 2: Sample sizes of each population in the common garden experiment (per treatment).

Sample sizes for laboratory populations	
Native	
Source	23
Caigual LP	21
Taylor LP	15
Tumbason LP	13
Introduction	
Lower Lalaja Intro	19
Upper Lalaja Intro	22
Caigual Intro	13
Taylor Intro	12

Table 3: Summary results from Procrustes ANOVA for field specimens.

	d.f.	SS	MS	R2	F	P
log(CS)	1	0.04736	0.047364	0.09803	77.4227	0.0001***
Population	7	0.19479	0.027827	0.40317	45.4866	0.0001***
log(CS):Population	7	0.00668	0.000955	0.01383	1.5603	0.0165*
Residuals	383	0.23431	0.000612	0.48496		
Total	398	0.48314				

Table 4: Post-hoc pairwise analysis for field specimens showing P-values of comparisons of mean Procrustes distance between populations.

	Source	UL	LL	IC	IT	CL	TL	TB
Source		0.0002	0.0001	0.0001	0.0001	0.0021	0.0060	0.0070
UL	0.0002		0.1049	0.0001	0.0001	0.0007	0.0001	0.0030
LL	0.0001	0.1049		0.0001	0.0001	0.0023	0.0001	0.0068
IC	0.0001	0.0001	0.0001		0.1565	0.0001	0.0001	0.0002
IT	0.0001	0.0001	0.0001	0.1565		0.0001	0.0001	0.0001
CL	0.0021	0.0007	0.0023	0.0001	0.0001		0.1140	0.5679
TL	0.0060	0.0001	0.0001	0.0001	0.0001	0.1140		0.1743
TB	0.0070	0.0030	0.0068	0.0001	0.0001	0.5679	0.1743	

Table 5: Post-hoc pairwise analysis for field specimens showing P-values of comparisons of allometric slopes (body size versus shape) between populations.

	Source	UL	LL	IC	IT	CL	TL	TB
Source		0.1393	0.0005	0.0533	0.0477	0.7934	0.3133	0.4577
UL	0.1393		0.0011	0.2478	0.9046	0.9987	0.7820	0.6580
LL	0.0005	0.0011		0.0067	0.0014	0.3024	0.0216	0.6927
IC	0.0533	0.2478	0.0067		0.2867	0.9603	0.1190	0.8783
IT	0.0477	0.9046	0.0014	0.2867		0.9665	0.4665	0.5361
CL	0.7934	0.9987	0.3024	0.9603	0.9665		0.9703	0.9768
TL	0.3133	0.7820	0.0216	0.1190	0.4665	0.9703		0.5300
TB	0.4577	0.6580	0.6927	0.8783	0.5361	0.9768	0.5300	

Table 6: Summary results from Procrustes ANOVA for laboratory specimens

	d.f.	SS	MS	R2	F	P
log(CS)	1	0.012009	0.0120086	0.05704	24.9607	0.0001***
Population	7	0.063860	0.0091228	0.30333	18.9624	0.0001***
Treatment	1	0.004635	0.0046351	0.02202	9.6343	0.0001***
log(CS):Population	7	0.003518	0.0005025	0.01671	1.0446	0.3830
log(CS):Treatment	1	0.000444	0.0004439	0.00211	0.9228	0.4670
Population:Treatment	7	0.005487	0.0007838	0.02606	1.293	0.0085**
log(CS):Population:Treatment	7	0.003189	0.0004556	0.01515	0.9469	0.5593
Residuals	244	0.117389	0.0004811	0.55759		
Total	275					

Table 7: Post-hoc pairwise analysis for laboratory specimens showing P-values of comparisons of mean Procrustes distance between populations under the predator cue (w/ cue) and no predator cue (w/o cue) treatments.

		Source		UL		LL		IC		IT		CL		TL		TB	
		w/ cue	w/o cue	w/ cue	w/o cue	w/ cue	w/o cue	w/ cue	w/o cue	w/ cue	w/o cue	w/ cue	w/o cue	w/ cue	w/o cue	w/ cue	w/o cue
Source	w/ cue		0.2330	0.5893	0.0001	0.0469	0.0032	0.0384	0.5190	0.5306	0.0003	0.0001	0.0001	0.0002	0.0004	0.0006	0.0001
	w/o cue	0.2330		0.2530	0.0098	0.0532	0.0191	0.0012	0.3833	0.1582	0.0011	0.0001	0.0001	0.0001	0.0016	0.0016	0.0001
UL	w/ cue	0.5893	0.2530		0.0338	0.9179	0.1349	0.0668	0.3709	0.5016	0.0038	0.0001	0.0002	0.0052	0.0015	0.0068	0.0002
	w/o cue	0.0001	0.0098	0.0338		0.0156	0.4520	0.0001	0.0053	0.0075	0.0001	0.0002	0.0004	0.0011	0.0068	0.0004	0.0001
LL	w/ cue	0.0469	0.0532	0.9179	0.0156		0.0481	0.0015	0.0720	0.1033	0.0001	0.0001	0.0001	0.0001	0.0001	0.0003	0.0001
	w/o cue	0.0032	0.0191	0.1349	0.4520	0.0481		0.0003	0.0326	0.0625	0.0028	0.0023	0.0134	0.0328	0.0335	0.0047	0.0006
IC	w/ cue	0.0384	0.0012	0.0668	0.0001	0.0015	0.0003		0.0676	0.6237	0.0003	0.0001	0.0001	0.0001	0.0002	0.0001	0.0001
	w/o cue	0.5190	0.3833	0.3709	0.0053	0.0720	0.0326	0.0676		0.5322	0.1182	0.0001	0.0004	0.0083	0.0077	0.0236	0.0001
IT	w/ cue	0.5306	0.1582	0.5016	0.0075	0.1033	0.0625	0.6237	0.5322		0.2713	0.0017	0.0073	0.0893	0.0312	0.0548	0.0026
	w/o cue	0.0003	0.0011	0.0038	0.0001	0.0001	0.0028	0.0003	0.1182	0.2713		0.0003	0.0014	0.0336	0.1084	0.0961	0.0012
CL	w/ cue	0.0001	0.0001	0.0001	0.0002	0.0001	0.0023	0.0001	0.0001	0.0017	0.0003		0.3675	0.0220	0.0655	0.0007	0.0004
	w/o cue	0.0001	0.0001	0.0002	0.0004	0.0001	0.0134	0.0001	0.0004	0.0073	0.0014	0.3675		0.1166	0.4076	0.0025	0.0016
TL	w/ cue	0.0002	0.0001	0.0052	0.0011	0.0001	0.0328	0.0001	0.0083	0.0893	0.0336	0.0220	0.1166		0.3259	0.0660	0.0248
	w/o cue	0.0004	0.0016	0.0015	0.0068	0.0001	0.0335	0.0002	0.0077	0.0312	0.1084	0.0655	0.4076	0.3259		0.0897	0.0975
TB	w/ cue	0.0006	0.0016	0.0068	0.0004	0.0003	0.0047	0.0001	0.0236	0.0548	0.0961	0.0007	0.0025	0.0660	0.0897		0.5806
	w/o cue	0.0001	0.0001	0.0002	0.0001	0.0001	0.0006	0.0001	0.0001	0.0026	0.0012	0.0004	0.0016	0.0248	0.0975	0.5806	

Figures

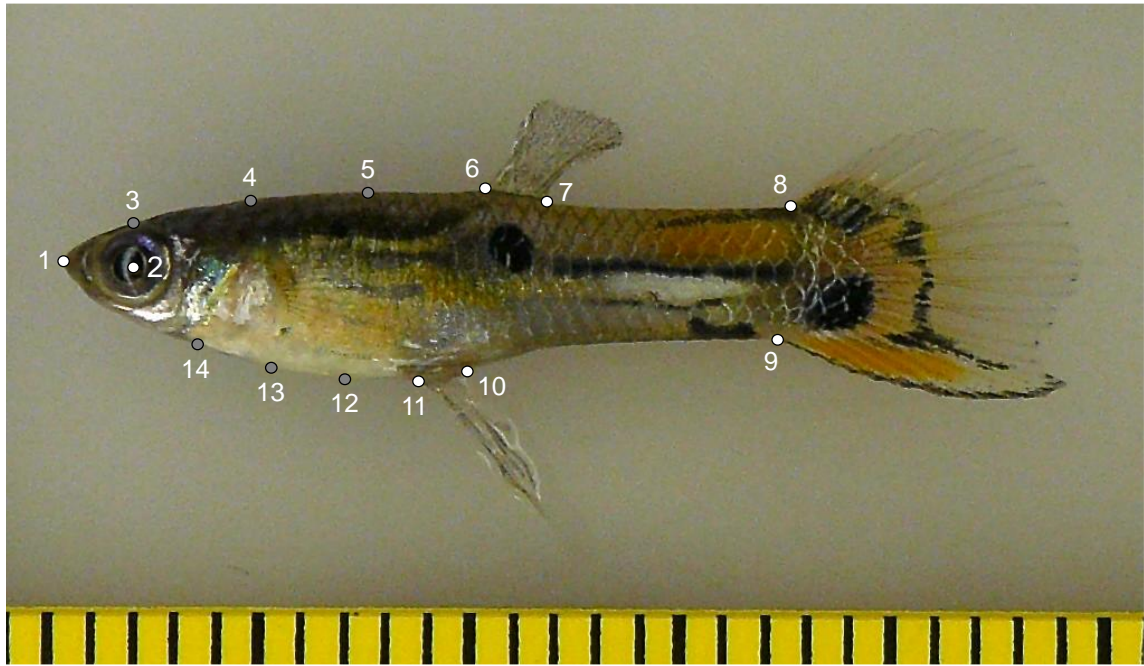


Fig 1: Landmarks used for geometric morphometric analysis. Fixed landmarks are represented by white circles (numbered 1, 2, 6–11) and semi-landmarks by grey circles (3–5, 12–14).

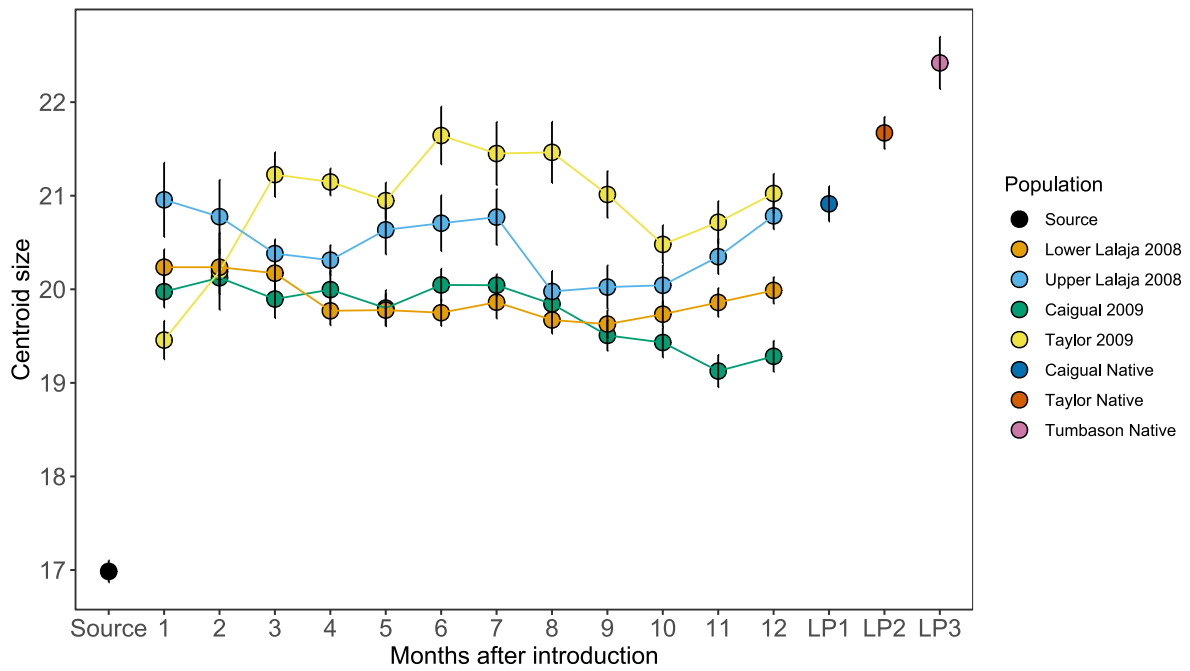


Fig 2: Mean centroid size (\pm SE) of the introduction guppies at each monthly interval. Means for the source and native low predation populations are also included for reference.

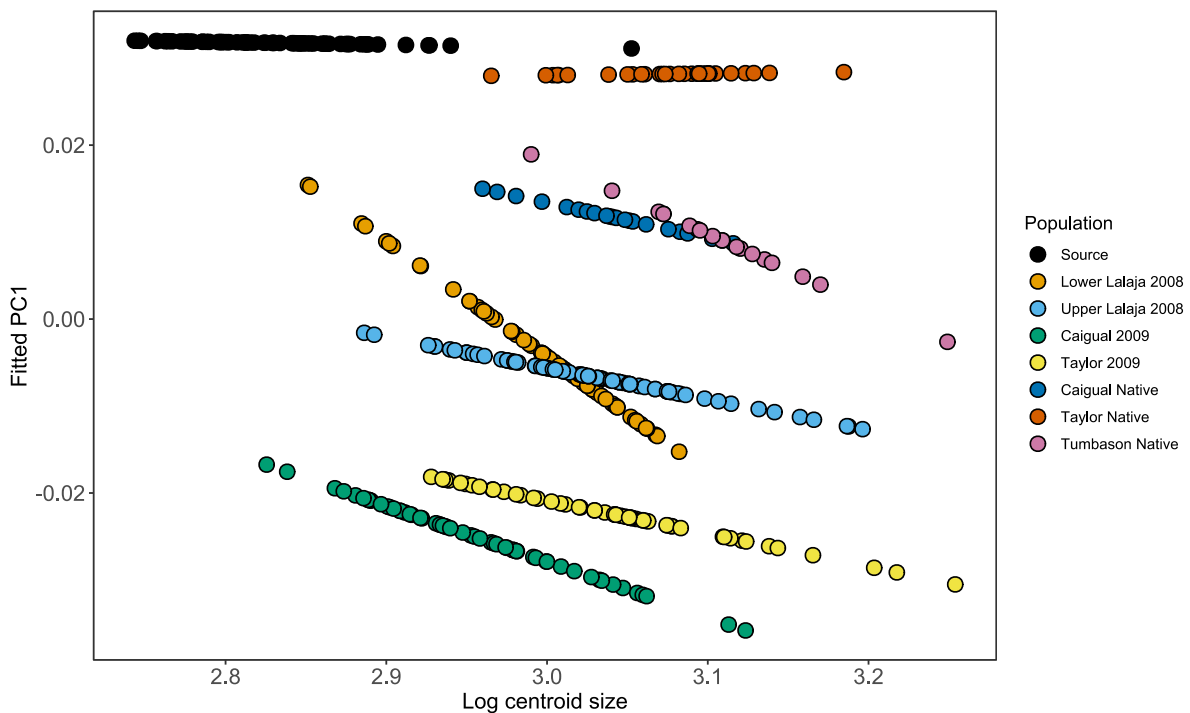


Fig 3: Slopes of the predicted value on PC1 for each field specimen versus their log centroid size, representing the allometric trends for each population.

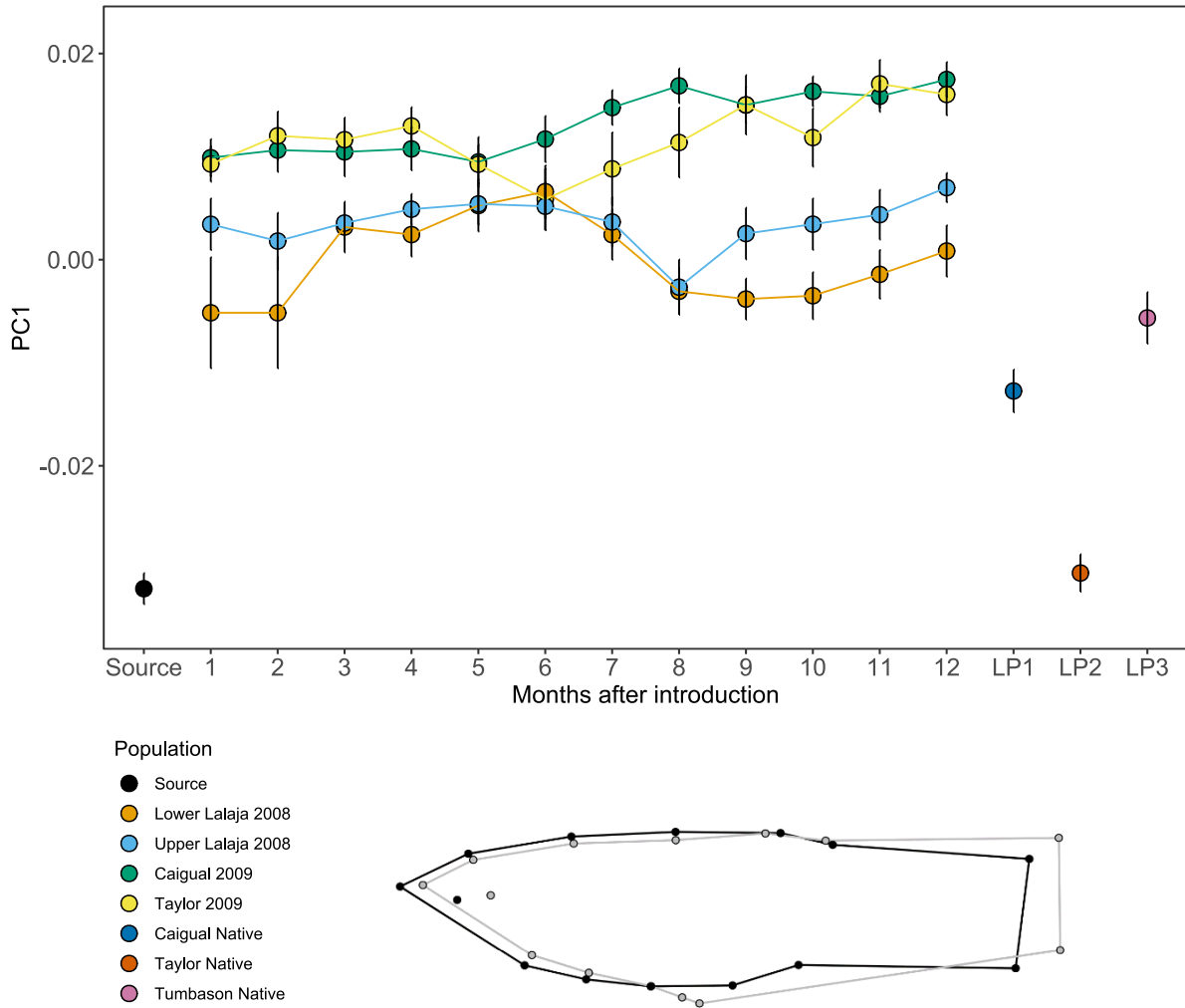


Fig 4: Mean PC1 scores (\pm SE) of the introduction guppies at each monthly interval. Means for the source and native low predation populations are also included for reference. Shape comparison between minimum and maximum PC1 values is shown below; the black outline represents shape at low PC1 values, and the grey outline represents shape at high PC1 values.

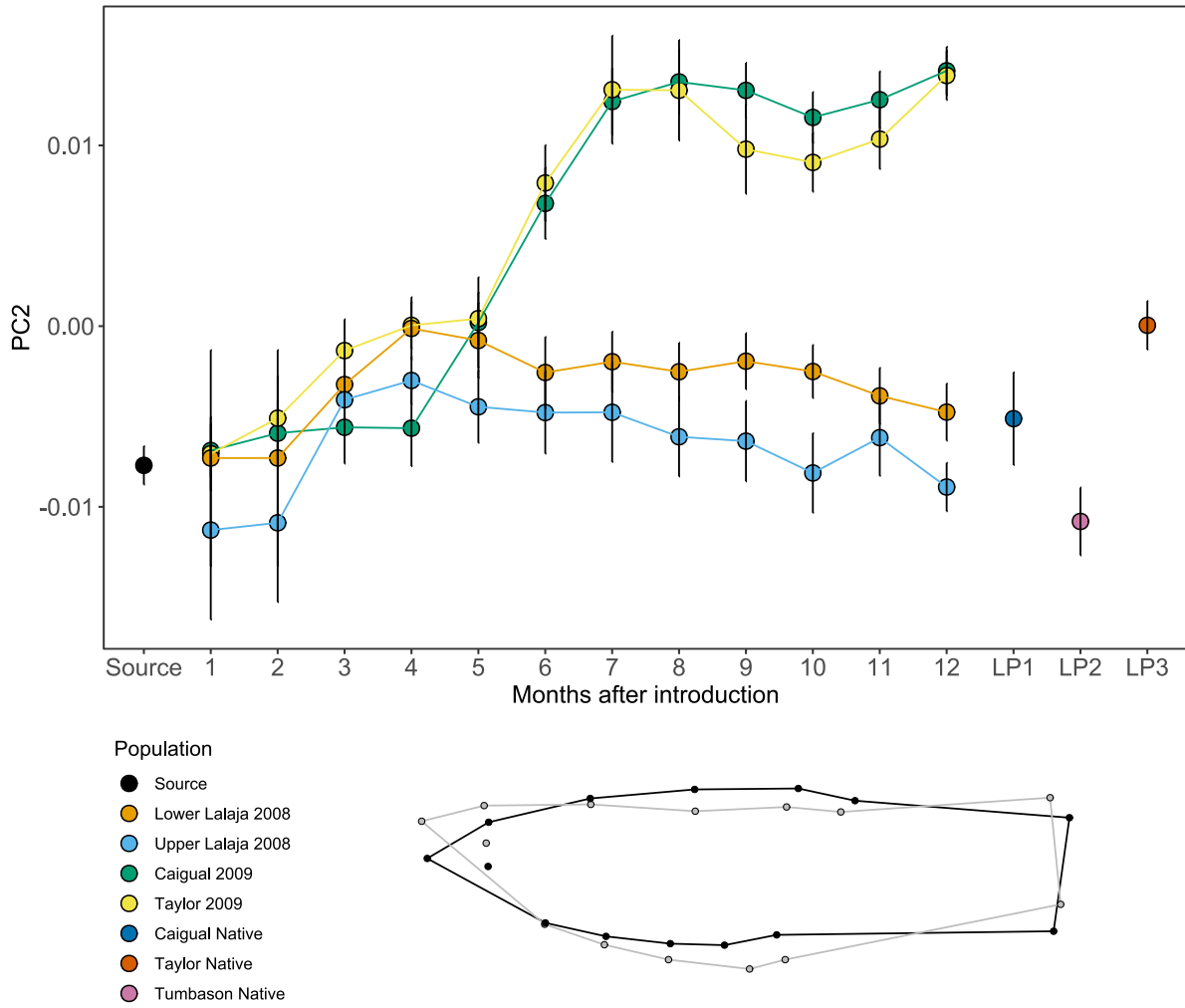


Fig 5: Mean PC2 scores (\pm SE) of the introduction guppies at each monthly interval. Means for the source and native low predation populations are also included for reference. Shape comparison between minimum and maximum PC2 values is shown below; the black outline represents shape at low PC2 values, and the grey outline represents shape at high PC2 values.

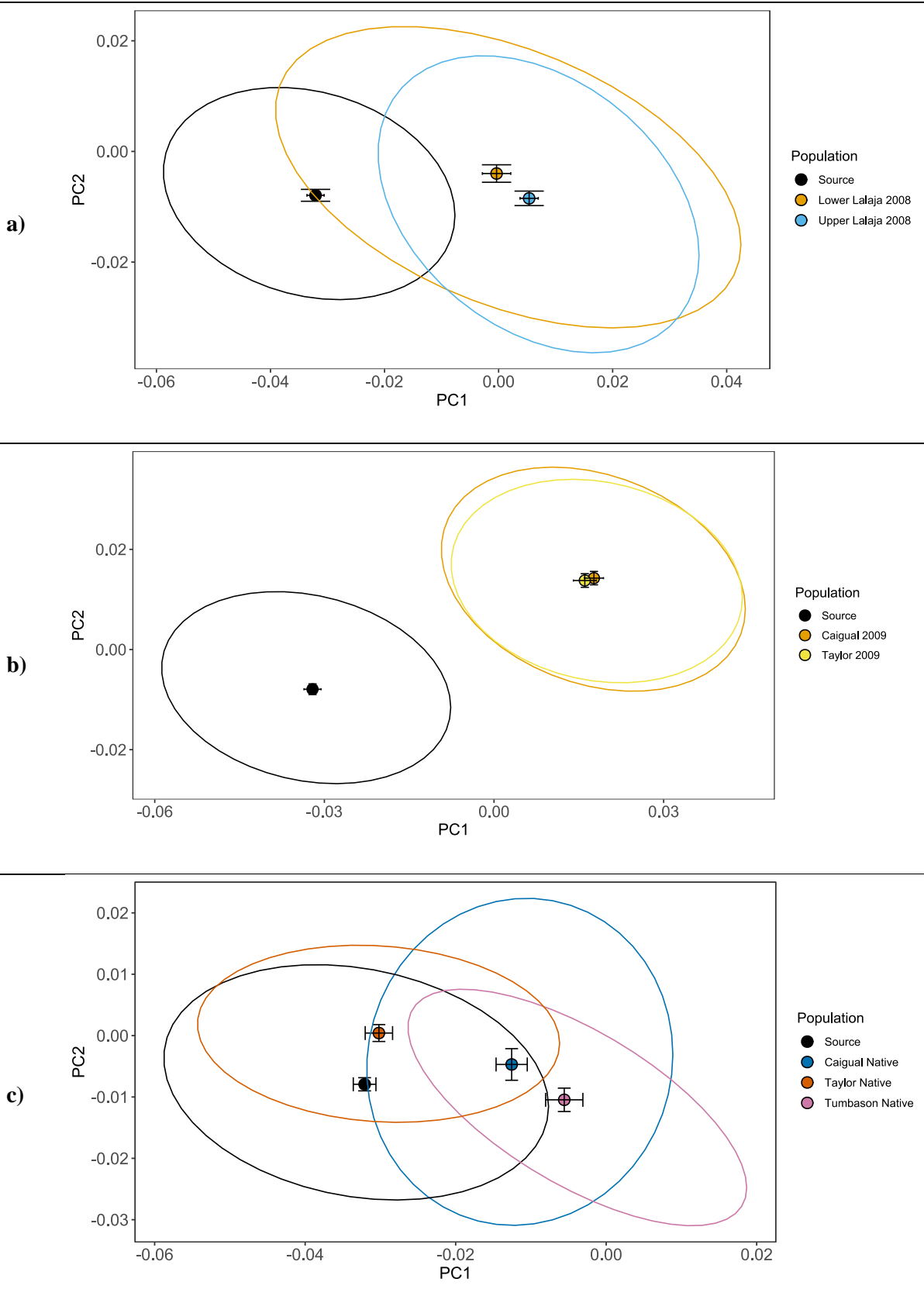


Fig 6: Mean PC1 and PC2 scores (+/- SE) for each population in the field with 95% confidence ellipses. Note that averages calculated for the introduction populations only included individuals

sampled at month 12. Visualisation has been split into three separate panels for ease of comparison between the source and a) the 2008 introduction populations (LL and UL), b) the 2009 introduction populations (IC and IT), and c) the native low predation populations (CL, TL, and TB).

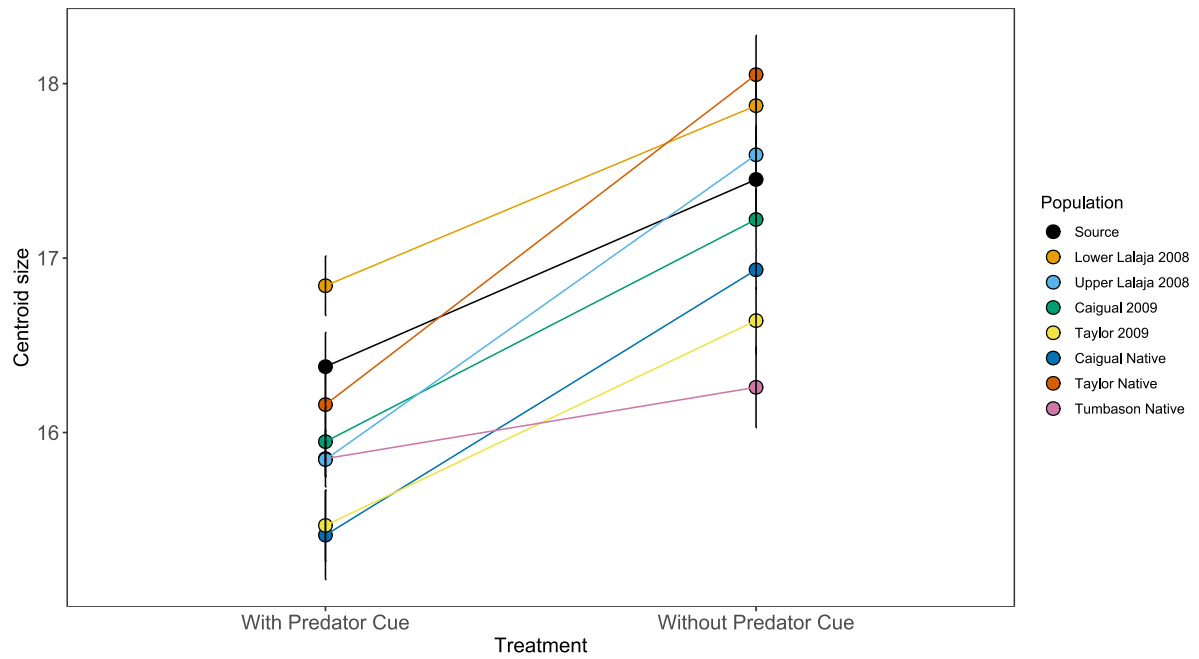


Fig 7: Mean centroid size (\pm SE) of guppies reared under common garden conditions in cue and no cue treatments, coloured by population.

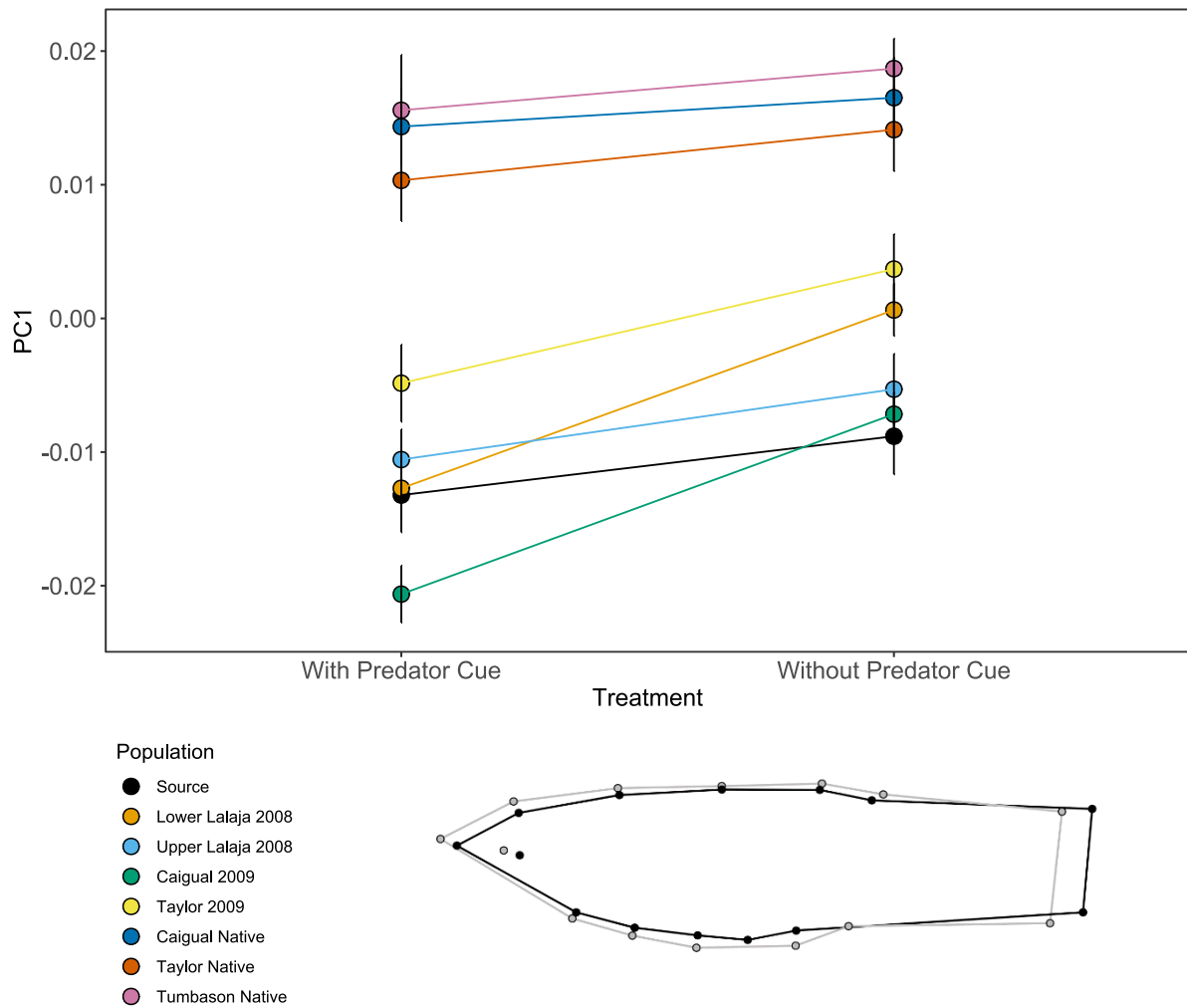


Fig 8: Mean PC1 scores (\pm SE) of guppies reared under common garden conditions in cue and no cue treatments, coloured by population. Shape comparison between minimum and maximum PC1 values is shown below; the black outline represents shape at low PC1 values, and the grey outline represents shape at high PC1 values.

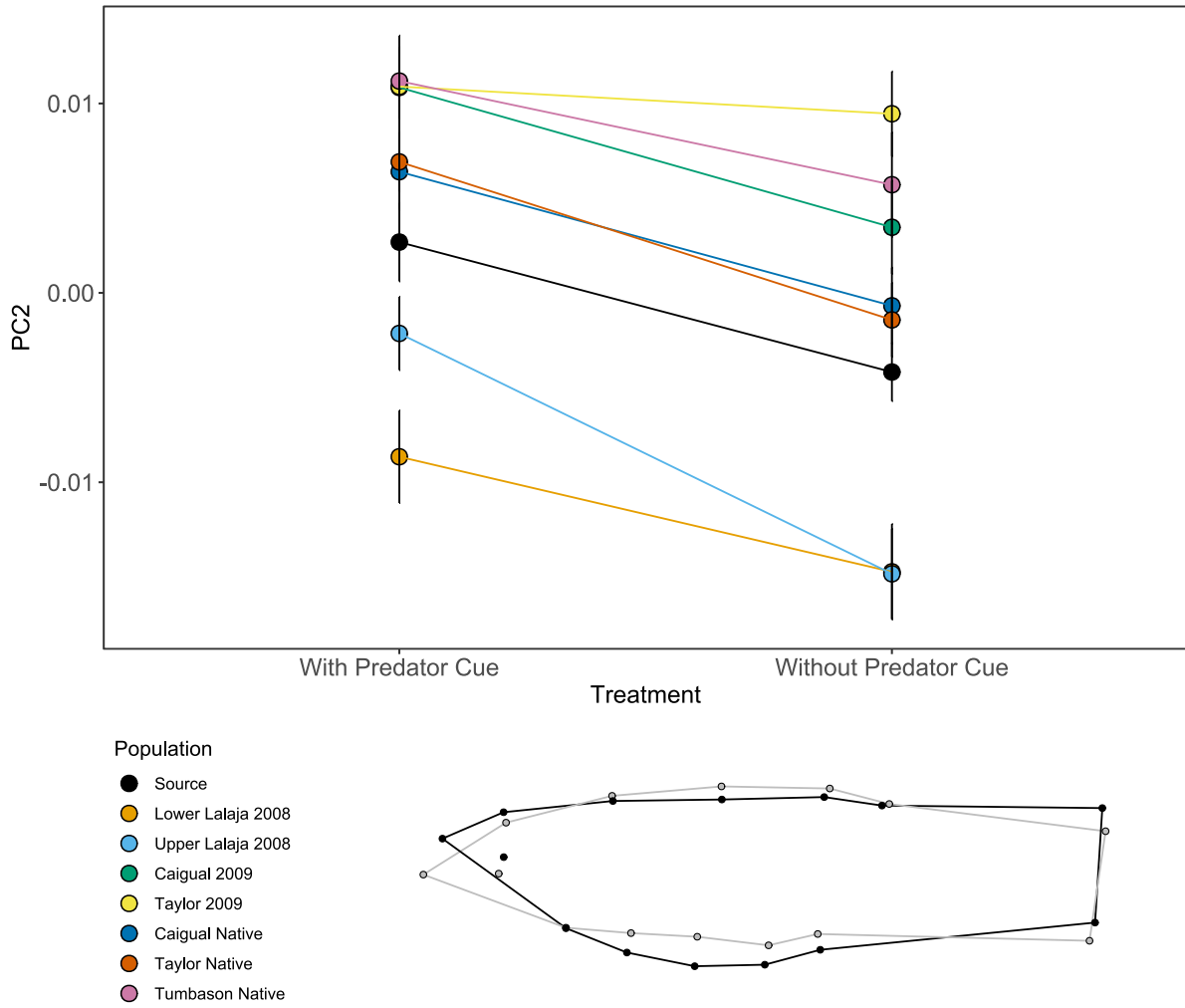


Fig 9: Mean PC2 scores (\pm SE) of guppies reared under common garden conditions in cue and no cue treatments, coloured by population. Shape comparison between minimum and maximum PC2 values is shown below; the black outline represents shape at low PC2 values, and the grey outline represents shape at high PC2 values.

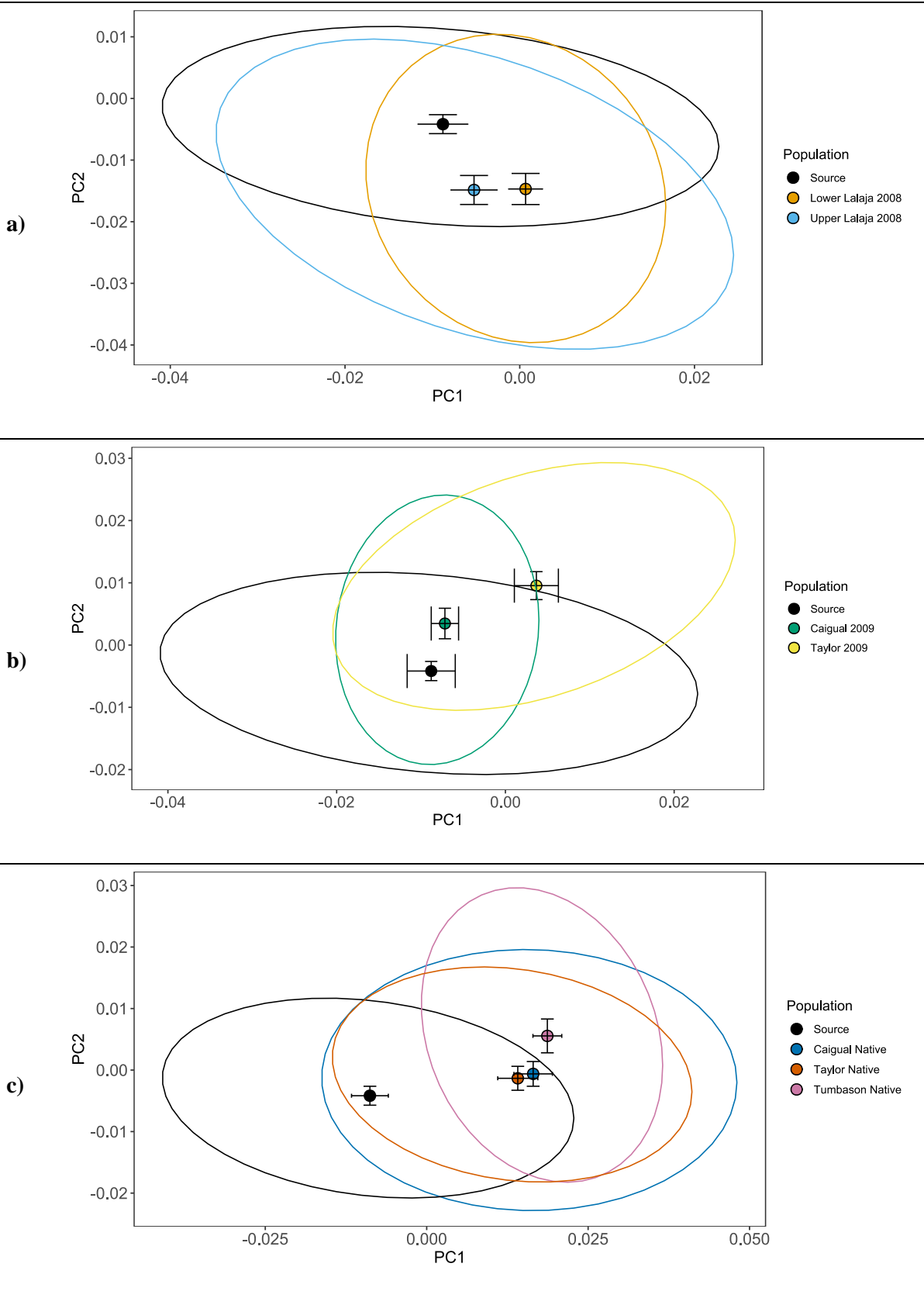


Fig 10: Mean PC1 and PC2 scores (+/- SE) for each population in the laboratory reared without a predator cue, with 95% confidence ellipses. Visualisation has been split into three separate panels for

ease of comparison between the source and a) the 2008 introduction populations (LL and UL), b) the 2009 introduction populations (IC and IT), and c) the native low predation populations (CL, TL, and TB).

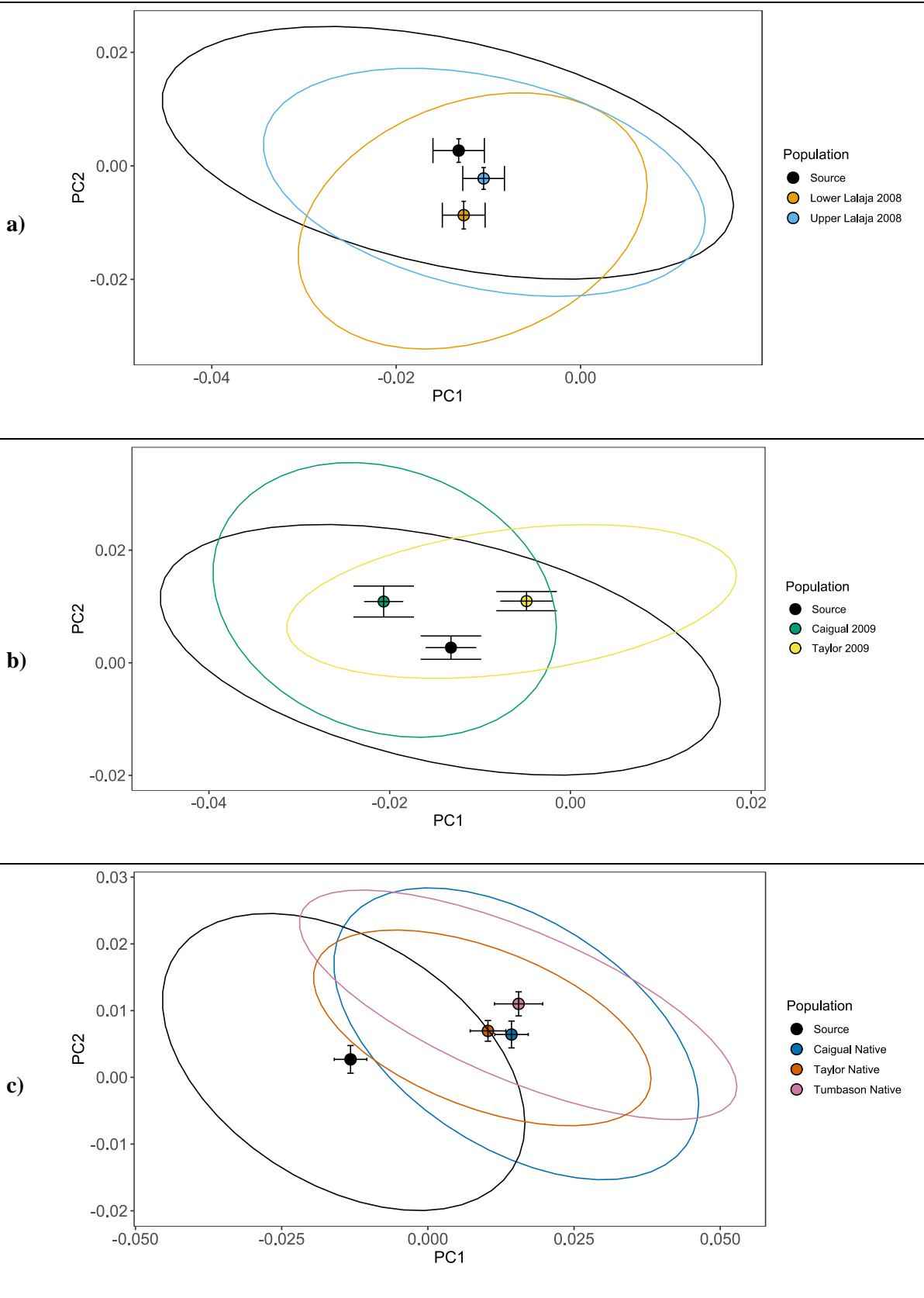


Fig 11: Mean PC1 and PC2 scores (+/- SE) for each population in the laboratory reared with a predator cue, with 95% confidence ellipses. Visualisation has been split into three separate panels for

ease of comparison between the source and a) the 2008 introduction populations (LL and UL), b) the 2009 introduction populations (IC and IT), and c) the native low predation populations (CL, TL, and TB).

Acknowledgements

I would like to thank my supervisor Cameron Ghalambor for his guidance, motivation, and optimism along this two-year journey. Thank you to Corey Handelsman who undertook these experiments and collected these extensive data which made this thesis possible. A special mention goes to my wonderful support network who motivated me along the way – Eleonora Jenisch, Serenoa Steiner, and Tuva Zeiner-Henriksen.

References

- Adams, D. C., & Otárola-Castillo, E. (2013). geomorph: An R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution*, 4(4), 393–399. <https://doi.org/10.1111/2041-210X.12035>
- Alexander, H. J., & Breden, F. (2004). Sexual isolation and extreme morphological divergence in the Cumana guppy: A possible case of incipient speciation. *Journal of Evolutionary Biology*, 17(6), 1238–1254. <https://doi.org/10.1111/j.1420-9101.2004.00788.x>
- Alexander, H. J., Taylor, J. S., Wu, S. S.-T., & Breden, F. (2006). Parallel evolution and vicariance in the guppy (*Poecilia reticulata*) over multiple spatial and temporal scales. *Evolution; International Journal of Organic Evolution*, 60(11), 2352–2369.
- Arnett, H. A., & Kinnison, M. T. (2016). Predator-induced phenotypic plasticity of shape and behavior: Parallel and unique patterns across sexes and species. *Current Zoology*, 63(4), 369–378. <https://doi.org/10.1093/cz/zow072>

- Arnold, S. J., Bürger, R., Hohenlohe, P. A., Ajie, B. C., & Jones, A. G. (2008). Understanding the evolution and stability of the G-matrix. *Evolution*, 62(10), 2451–2461.
<https://doi.org/10.1111/j.1558-5646.2008.00472.x>
- Bassar, R. D., Marshall, M. C., López-Sepulcre, A., Zandonà, E., Auer, S. K., Travis, J., Pringle, C. M., Flecker, A. S., Thomas, S. A., Fraser, D. F., & Reznick, D. N. (2010). Local adaptation in Trinidadian guppies alters ecosystem processes. *Proceedings of the National Academy of Sciences*, 107(8), 3616–3621. <https://doi.org/10.1073/pnas.0908023107>
- Bookstein, F. L. (1997). *Morphometric tools for landmark data: Geometry and biology* (1st pbk. ed). Cambridge University Press.
- Burns, J. G., Di Nardo, P., & Rodd, F. H. (2009). The role of predation in variation in body shape in guppies *Poecilia reticulata*: A comparison of field and common garden phenotypes. *Journal of Fish Biology*, 75(6), 1144–1157. <https://doi.org/10.1111/j.1095-8649.2009.02314.x>
- Cheverud, J. M. (1988). A comparison of genetic and phenotypic correlations. *Evolution*, 42(5), 958–968. <https://doi.org/10.1111/j.1558-5646.1988.tb02514.x>
- Conover, D. O., & Schultz, E. T. (1995). Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology & Evolution*, 10(6), 248–252.
[https://doi.org/10.1016/S0169-5347\(00\)89081-3](https://doi.org/10.1016/S0169-5347(00)89081-3)
- Draghi, J. A., & Whitlock, M. C. (2012). Phenotypic plasticity facilitates mutational variance, genetic variance, and evolvability along the major axis of environmental variation: phenotypic plasticity facilitates mutational variance, genetic variance, and evolvability. *Evolution*, 66(9), 2891–2902. <https://doi.org/10.1111/j.1558-5646.2012.01649.x>

- Dussault, G. V., & Kramer, D. L. (1981). Food and feeding behavior of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Canadian Journal of Zoology*, 59(4), 684–701.
<https://doi.org/10.1139/z81-098>
- Dzikowski, R., Hulata, G., Harpaz, S., & Karplus, I. (2004). Inducible reproductive plasticity of the guppy *Poecilia reticulata* in response to predation cues. *Journal of Experimental Zoology*, 301(9), 776–782. <https://doi.org/10.1002/jez.a.61>
- Fu, S. J., Cao, Z. D., Yan, G. J., Fu, C., & Pang, X. (2013). Integrating environmental variation, predation pressure, phenotypic plasticity and locomotor performance. *Oecologia*, 173(2), 343–354. <https://doi.org/10.1007/s00442-013-2626-7>
- Ghalambor, C. K., Hoke, K. L., Ruell, E. W., Fischer, E. K., Reznick, D. N., & Hughes, K. A. (2015). Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature*, 525(7569), 372–375. <https://doi.org/10.1038/nature15256>
- Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, 21(3), 394–407. <https://doi.org/10.1111/j.1365-2435.2007.01283.x>
- Gibert, P., Debat, V., & Ghalambor, C. K. (2019). Phenotypic plasticity, global change, and the speed of adaptive evolution. *Current Opinion in Insect Science*, 35, 34–40.
<https://doi.org/10.1016/j.cois.2019.06.007>
- Goodall, C. (1991). Procrustes methods in the statistical analysis of shape. *Journal of the Royal Statistical Society: Series B (Methodological)*, 53(2), 285–321.
<https://doi.org/10.1111/j.2517-6161.1991.tb01825.x>

- Grether, G. F., Millie, D. F., Bryant, M. J., Reznick, D. N., & Mayea, W. (2001). Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology*, 82(6), 1546–1559. [https://doi.org/10.1890/0012-9658\(2001\)082\[1546:RFCCRA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[1546:RFCCRA]2.0.CO;2)
- Gu, L., Xu, Y., Yang, T., Qin, S., Zhang, L., Sun, Y., Huang, Y., & Yang, Z. (2021). Predator-induced allometric changes in the tail spine length of *Daphnia*: A distinct resource allocation strategy. *Journal of Plankton Research*, 43(6), 884–893. <https://doi.org/10.1093/plankt/fbab063>
- Handelsman, C. A., Broder, E. D., Dalton, C. M., Ruell, E. W., Myrick, C. A., Reznick, D. N., & Ghalambor, C. K. (2013). Predator-Induced Phenotypic Plasticity in Metabolism and Rate of Growth: Rapid Adaptation to a Novel Environment. *Integrative and Comparative Biology*, 53(6), 975–988. <https://doi.org/10.1093/icb/ict057>
- Handelsman, C. A., Ruell, E. W., Torres-Dowdall, J., & Ghalambor, C. K. (2014). Phenotypic plasticity changes correlations of traits following experimental introductions of Trinidadian guppies (*Poecilia reticulata*). *Integrative and Comparative Biology*, 54(5), 794–804. <https://doi.org/10.1093/icb/icu112>
- Hendry, A. P., Kelly, M. L., Kinnison, M. T., & Reznick, D. N. (2006). Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies. *Journal of Evolutionary Biology*, 19(3), 741–754. <https://doi.org/10.1111/j.1420-9101.2005.01061.x>
- James Rohlf, F., & Marcus, L. F. (1993). A revolution morphometrics. *Trends in Ecology & Evolution*, 8(4), 129–132. [https://doi.org/10.1016/0169-5347\(93\)90024-J](https://doi.org/10.1016/0169-5347(93)90024-J)
- Januszkiewicz, A. J., & Robinson, B. W. (2007). Divergent walleye (*Sander vitreus*) mediated inducible defenses in the centrarchid pumpkinseed sunfish (*Lepomis gibbosus*). *Biological*

Journal of the Linnean Society, 90(1), 25–36. <https://doi.org/10.1111/j.1095-8312.2007.00708.x>

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, 7(12), 1225–1241. <https://doi.org/10.1111/j.1461-0248.2004.00684.x>

Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology*, 22(7), 1435–1446. <https://doi.org/10.1111/j.1420-9101.2009.01754.x>

Lande, R., & Arnold, S. J. (1983). The measurement of selection on correlated characters. *Evolution*, 37(6), 1210–1226. <https://doi.org/10.1111/j.1558-5646.1983.tb00236.x>

Langerhans, R. B., Layman, C. A., Shokrollahi, A. M., & DeWitt, T. J. (2004). Predator-driven phenotypic diversification in *Gambusia affinis*. *Evolution*, 58(10), 2305–2318. <https://doi.org/10.1111/j.0014-3820.2004.tb01605.x>

Langerhans, R. B., & Reznick, D. N. (2009). Ecology and evolution of swimming performance in fishes: Predicting evolution with biomechanics. In *Fish locomotion: An etho-ecological perspective* (1st ed., pp. 200–248). CRC Press.

Liley, N. R., & Seghers, B. H. (1975). Factors affecting the morphology and behaviour of guppies in Trinidad. In *Function and evolution in behaviour* (pp. 92–118). Oxford University Press.

Magurran, A. E. (2005). *Evolutionary ecology: The Trinidadian guppy*. Oxford University Press.

Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. *Evolutionary Applications*, 7(1), 1–14. <https://doi.org/10.1111/eva.12137>

Noble, D. W. A., Radersma, R., & Uller, T. (2019). Plastic responses to novel environments are biased towards phenotype dimensions with high additive genetic variation. *Proceedings of*

the National Academy of Sciences, 116(27), 13452–13461.

<https://doi.org/10.1073/pnas.1821066116>

Nowlin, W. H., Drenner, R. W., Guckenberger, K. R., Laudén, M. A., Alonso, G. T., Fennell, J. E., & Smith, J. L. (2006). Gape limitation, prey size refuges and the top–down impacts of piscivorous largemouth bass in shallow pond ecosystems. *Hydrobiologia*, 563(1), 357–369.

<https://doi.org/10.1007/s10750-006-0024-4>

Paenke, I., Sendhoff, B., & Kawecki, T. J. (2007). Influence of plasticity and learning on evolution under directional selection. *The American Naturalist*, 170(2), E47–E58.

<https://doi.org/10.1086/518952>

Pakkasmaa, S., & Piironen, J. (2001). Morphological differentiation among local trout (*Salmo trutta*) populations. *Biological Journal of the Linnean Society*, 72(2), 231–239.

<https://doi.org/10.1111/j.1095-8312.2001.tb01313.x>

Preisser, E. L., & Orrock, J. L. (2012). The allometry of fear: Interspecific relationships between body size and response to predation risk. *Ecosphere*, 3(9), art77.

<https://doi.org/10.1890/ES12-00084.1>

Price, T. D., Qvarnström, A., & Irwin, D. E. (2003). The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1523), 1433–1440. <https://doi.org/10.1098/rspb.2003.2372>

Reznick, D. (1982). The impact of predation on life history evolution in Trinidadian guppies: Genetic basis of observed life history patterns. *Evolution*, 36(6), 1236–1250.

<https://doi.org/10.1111/j.1558-5646.1982.tb05493.x>

Reznick, D. A., Bryga, H., & Endler, J. A. (1990). Experimentally induced life-history evolution in a natural population. *Nature*, 346(6282), 357–359. <https://doi.org/10.1038/346357a0>

- Reznick, D., Butler IV, M. J., & Rodd, H. (2001). Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *The American Naturalist*, 157(2), 126–140. <https://doi.org/10.1086/318627>
- Reznick, D., & Endler, J. A. (1982). The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution*, 36(1), 160. <https://doi.org/10.2307/2407978>
- Reznick, D. N., Bassar, R. D., Handelsman, C. A., Ghalambor, C. K., Arendt, J., Coulson, T., Potter, T., Ruell, E. W., Torres-Dowdall, J., Bentzen, P., & Travis, J. (2019). Eco-evolutionary feedbacks predict the time course of rapid life-history evolution. *The American Naturalist*, 194(5), 671–692. <https://doi.org/10.1086/705380>
- Reznick, D. N., & Bryga, H. (1987). Life-history evolution in guppies (*Poecilia reticulata*): 1. Phenotypic and genetic changes in an introduction experiment. *Evolution*, 41(6), 1370–1385. <https://doi.org/10.1111/j.1558-5646.1987.tb02474.x>
- Reznick, D. N., & Bryga, H. A. (1996). Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). V. Genetic basis of parallelism in life histories. *The American Naturalist*, 147(3), 339–359. <https://doi.org/10.1086/285855>
- Reznick, D. N., Shaw, F. H., Rodd, F. H., & Shaw, R. G. (1997). Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science*, 275(5308), 1934–1937. <https://doi.org/10.1126/science.275.5308.1934>
- Robinson, B. W., & Parsons, K. J. (2002). Changing times, spaces, and faces: Tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 59(11), 1819–1833. <https://doi.org/10.1139/f02-144>

- Robinson, B. W., & Wilson, D. S. (1995). Experimentally induced morphological diversity in Trinidadian guppies (*Poecilia reticulata*). *Copeia*, 1995(2), 294.
<https://doi.org/10.2307/1446893>
- Roff, D. A. (1995). The estimation of genetic correlations from phenotypic correlations: A test of Cheverud's conjecture. *Heredity*, 74(5), 481–490. <https://doi.org/10.1038/hdy.1995.68>
- Rohlf, F. J., & Slice, D. (1990). Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology*, 39(1), 40.
<https://doi.org/10.2307/2992207>
- Sakata, Y., Utsumi, S., Craig, T. P., Itami, J. K., Ikemoto, M., & Ohgushi, T. (2020). Environmentally triggered variability in the genetic variance–covariance of herbivory resistance of an exotic plant *Solidago altissima*. *Ecology and Evolution*, 10(6), 3103–3111.
<https://doi.org/10.1002/ece3.6130>
- Schlichting, C., & Pigliucci, M. (1998). *Phenotypic evolution: A reaction norm perspective*. Sinauer.
- Schluter, D. (1996). Adaptive radiation along genetic lines of least resistance. *Evolution*, 50(5), 1766. <https://doi.org/10.2307/2410734>
- Schluter, D., & McPhail, J. D. (1992). Ecological character displacement and speciation in sticklebacks. *The American Naturalist*, 140(1), 85–108. <https://doi.org/10.1086/285404>
- Seghers, B. H. (1973). *Analysis of geographic variation in the antipredator adaptations of the guppy: Poecilia reticulata*. University of British Columbia.
- Sgrò, C. M., & Hoffmann, A. A. (2004). Genetic correlations, tradeoffs and environmental variation. *Heredity*, 93(3), 241–248. <https://doi.org/10.1038/sj.hdy.6800532>

- Sommer, R. J. (2020). Phenotypic plasticity: From theory and genetics to current and future challenges. *Genetics*, 215(1), 1–13. <https://doi.org/10.1534/genetics.120.303163>
- Svanbäck, R., & Eklöv, P. (2002). Effects of habitat and food resources on morphology and ontogenetic growth trajectories in perch. *Oecologia*, 131(1), 61–70. <https://doi.org/10.1007/s00442-001-0861-9>
- Torres-Dowdall, J., Handelsman, C. A., Reznick, D. N., & Ghalambor, C. K. (2012). Local adaptation and the evolution of phenotypic plasticity in Trinidadian guppies (*Poecilia reticulata*): Adaptation and the evolution of phenotypic plasticity. *Evolution*, 66(11), 3432–3443. <https://doi.org/10.1111/j.1558-5646.2012.01694.x>
- Via, S., & Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution*, 39(3), 505–522. <https://doi.org/10.1111/j.1558-5646.1985.tb00391.x>
- Videler, J. J. (1993). *Fish swimming* (1st ed). Chapman & Hall.
- Walker, J. A. (2010). An integrative model of evolutionary covariance: A symposium on body shape in fishes. *Integrative and Comparative Biology*, 50(6), 1051–1056. <https://doi.org/10.1093/icb/icq014>
- Webb, P. W. (1978). Fast-start performance and body form in seven species of teleost fish. *Journal of Experimental Biology*, 74(1), 211–226. <https://doi.org/10.1242/jeb.74.1.211>
- Webb, P. W. (1982). Locomotor patterns in the evolution of actinopterygian fishes. *American Zoologist*, 22(2), 329–342. <https://doi.org/10.1093/icb/22.2.329>
- Webb, P. W. (1984). Body form, locomotion and foraging in aquatic vertebrates. *American Zoologist*, 24(1), 107–120. <https://doi.org/10.1093/icb/24.1.107>
- Williams, G. C. (1966). *Adaptation and natural selection: A critique of some current evolutionary thought* (Facsim. ed., with new pref.). Princeton University Press.

- Williams, T. J., Johnson, J. B., & Belk, M. C. (2017). Interaction between predation environment and diet constrains body shape in Utah chub, *Gila atraria* (Cypriniformes: Cyprinidae). *Biological Journal of the Linnean Society*, *122*(1), 147–156.
<https://doi.org/10.1093/biolinnea/blx050>
- Willing, E.-M., Bentzen, P., Van Oosterhout, C., Hoffmann, M., Cable, J., Breden, F., Weigel, D., & Dreyer, C. (2010). Genome-wide single nucleotide polymorphisms reveal population history and adaptive divergence in wild guppies. *Molecular Ecology*, *19*(5), 968–984.
<https://doi.org/10.1111/j.1365-294X.2010.04528.x>
- Wimberger, P. H. (1992). Plasticity of fish body shape. The effects of diet, development, family and age in two species of *Geophagus* (Pisces: Cichlidae). *Biological Journal of the Linnean Society*, *45*(3), 197–218. <https://doi.org/10.1111/j.1095-8312.1992.tb00640.x>
- Wood, C. W., & Brodie, E. D. (2015). Environmental effects on the structure of the G-matrix. *Evolution*, *69*(11), 2927–2940. <https://doi.org/10.1111/evo.12795>
- Wood, C. W., & Brodie, E. D. (2016). Evolutionary response when selection and genetic variation covary across environments. *Ecology Letters*, *19*(10), 1189–1200.
<https://doi.org/10.1111/ele.12662>
- Wund, M. A., Valena, S., Wood, S., & Baker, J. A. (2012). Ancestral plasticity and allometry in threespine stickleback reveal phenotypes associated with derived, freshwater ecotypes. *Biological Journal of the Linnean Society*, *105*(3), 573–583. <https://doi.org/10.1111/j.1095-8312.2011.01815.x>
- Zandonà, E., Auer, S. K., Kilham, S. S., Howard, J. L., López-Sepulcre, A., O'Connor, M. P., Bassar, R. D., Osorio, A., Pringle, C. M., & Reznick, D. N. (2011). Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies: Diet

correlates with life histories in guppy. *Functional Ecology*, 25(5), 964–973.

<https://doi.org/10.1111/j.1365-2435.2011.01865.x>

Zelditch, M. (Ed.). (2004). *Geometric morphometrics for biologists: A primer*. Elsevier Academic Press.



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