

Morphological Divergence of Native and Recently Established Populations of White Sands Pupfish (*Cyprinodon tularosa*)

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We used landmark-based geometric morphometric methods to describe patterns of body shape variation and shape covariation with size among populations of the threatened White Sands Pupfish (*Cyprinodon tularosa*), a species that occurs in dissimilar aquatic habitats. White Sands Pupfish populations include two genetically distinct, native populations that have been historically isolated in Salt Creek, a saline river, and Malpais Spring, a brackish spring. In addition, two populations were established approximately 30 years before this study by translocation of fish from Salt Creek to Lost River (a saline river) and Mound Spring (a brackish spring). We found significant body shape variation among populations and between males and females. Body shapes were more slender for females than for males and more slender for saline river populations than brackish spring populations. Introductions of pupfish to new habitats resulted in significant departures in body shape and shape allometry from the native Salt Creek population. Shape divergence was more pronounced for the Mound Spring population, which is consistent with a greater change in abiotic conditions. Although Mound Spring pupfish, like Malpais Spring pupfish, were more deep-bodied than saline river pupfish, differences in body shape and the level of sexual dimorphism were significant between the two brackish spring populations, indicating that deep-bodied shapes may be achieved from different anatomical configurations. The significant shape divergence of introduced populations warrants consideration for the conservation of this rare species, as creation of refuge populations for native stocks is a current management strategy.

PUPFISHES of the genus *Cyprinodon* represent a group of fishes known for their ability to tolerate variable environmental conditions in desert aquatic habitats of North America. Morphological diversification of inland species is thought to be associated with isolation of populations in ecologically disparate, remnant aquatic habitats following desiccation of large Pleistocene lakes (Miller, 1981). Early accounts of species descriptions were based chiefly on differences in morphometric and meristic data (e.g., Hubbs, 1932; Miller, 1943, 1948). These early papers represent exhaustive studies of species descriptions, where detailed morphometric and meristic measurements were compared, trait-by-trait. However, intraspecific ecological morphology comparisons have not been considered in detail for this group of fishes, despite their renowned ability to survive in a variety of aquatic desert habitats.

Recent advances in morphometric analytical techniques have provided statistically powerful

methods for the analysis of shape (e.g., Rohlf and Slice, 1990). These techniques, inclusively named geometric morphometrics (Rohlf and Marcus 1993; Adams et al., 2004), describe the spatial arrangement of anatomical features of organisms, providing statistical (Rohlf, 1999) and visual (e.g., Caldecutt and Adams, 1998; Adams and Rohlf, 2000; Rüber and Adams, 2001) advantages to traditional approaches based on linear distance measures. These techniques have been used increasingly over the past decade with morphological data from fishes for studies of phylogenetics and species descriptions (e.g., Corti and Crosetti, 1996; Cavalcanti et al., 1999; Douglas et al., 2001), ontogenetic allometry (e.g., Walker, 1993; Hood and Heins, 2000; Gallo-Da-Silva et al., 2002), trophic morphology (e.g., Caldecutt and Adams, 1999; Rüber and Adams, 2001), ecological morphology (e.g., Corti et al., 1996; Walker, 1996, 1997), and osteology (e.g., Loy et al., 1999, 2001). Geometric morphometric methods allow fine-scale

assessment of shape differences and, therefore, could be valuable for discerning patterns of intraspecific morphological variation.

In this study, we use landmark-based, geometric morphometric methods to describe patterns of body shape variation and covariation with fish size among populations of White Sands Pupfish (*Cyprinodon tularosa*), which occur in dissimilar environments in the Tularosa Basin in southern New Mexico. The White Sands Pupfish is listed as threatened by New Mexico and composed of two native and two recently introduced populations (Pittenger and Springer, 1999). The native populations occur in brackish spring (Malpais Spring) and saline river (Salt Creek) habitats and have been isolated presumably since the desiccation of the Pleistocene Lake Otero (Miller and Echelle, 1975; Pittenger and Springer, 1999). Miller and Echelle (1975) noted morphological and meristic differences between the two native populations. Salt Creek Pupfish had a more slender body and scales that were smaller and more numerous. In addition, Salt Creek Pupfish had less scalation of the belly. These differences did not warrant subspecies designation, however, according to the authors. Nevertheless, genetic distinction of the two native populations has been reported (Stockwell et al., 1998; Iyengar et al., 2004) based on fixed or nearly fixed differences in allele frequencies of allozymes and microsatellites.

The two introduced populations also occur in a brackish spring (Mound Spring) and a saline river (Lost River). These populations were established circa 1970 (Pittenger and Springer, 1999) from translocation of Salt Creek fish (Stockwell et al., 1998). They are not genetically differentiated from the Salt Creek population based on genetic distances using allozyme and microsatellite data (Stockwell et al., 1998), but the introductions resulted in shifts in allele frequency from the Salt Creek population and loss of some alleles at various microsatellite markers for both introduced populations (Stockwell and Mulvey, 1998; Stockwell et al., 1998; Miller, 2001).

The creation of refuge populations for native genetic stocks is a current management strategy for the White Sands Pupfish. The introduction of the Lost River and Mound Spring populations approximately three decades before this study has served as a source of ongoing research to evaluate the ecological and evolutionary implications associated with creating refuge populations. The purpose of this study was to consider morphological divergence of pupfish populations introduced to new environments. Our objectives were to describe body shape variation

among populations, shape covariation with pupfish size (shape allometry), and sexual dimorphism in body shape, to gain an understanding of pupfish ecological morphology.

MATERIALS AND METHODS

We examined 393 specimens of White Sands Pupfish collected from the four populations ($N = 49$ -50 females and $N = 45$ -50 males from each population). Descriptions of collection sites are described in detail by Collyer (2003). Nonmetric multidimensional scaling revealed that White Sands Pupfish habitats can be distinctly described as brackish springs (BS = Malpais Spring and Mound Spring) or saline rivers (SR = Salt Creek and Lost River) based on abiotic factors including temperature, salinity, and water flow (Collyer, 2003).

Fish collection and landmark acquisition.—Adult pupfish were used in this study to reduce the amount of intrapopulation shape variation based on ontogeny. Pupfish were collected from 26 May to 8 June 2001, at the middle sections of Lost River (26-27 May) and Salt Creek (30 May), the springhead and outflow marsh of Malpais Spring (4-5 June), and the upper pond of Mound Spring (8 June). Fish were live-captured with unbaited minnow traps placed at depths less than one meter at Lost River and Salt Creek, with beach seines at Mound Spring, and with a combination of the two techniques at Malpais Spring. Fish were transported live to a research laboratory at Holloman Air Force Base, sacrificed in approximately 500 mg/L tricaine methanesulfonate (MS 222; Summerfelt and Smith, 1990), separated into female and male groups, and placed into ice baths. Fish were patted dry, labeled, and photographed within two hours of sacrifice.

Digital photographs were captured with a Minolta RD-175(r) digital camera mounted on a photography table approximately 0.25 m directly above the table surface. The left lateral surface of each specimen was photographed using flash lighting. Thirteen landmarks on each specimen (Fig. 1) were digitized using TPSDIG software, version 1.31 (F. J. Rohlf, 2001, unpubl.). These landmarks represent anatomical homologs that could be easily identified for each specimen and provide quantitative information for describing shape.

Shape analysis.—We used landmark-based geometric morphometrics techniques to quantify body shape (Rohlf and Marcus, 1993; Adams et al., 2004). Geometric morphometric methods

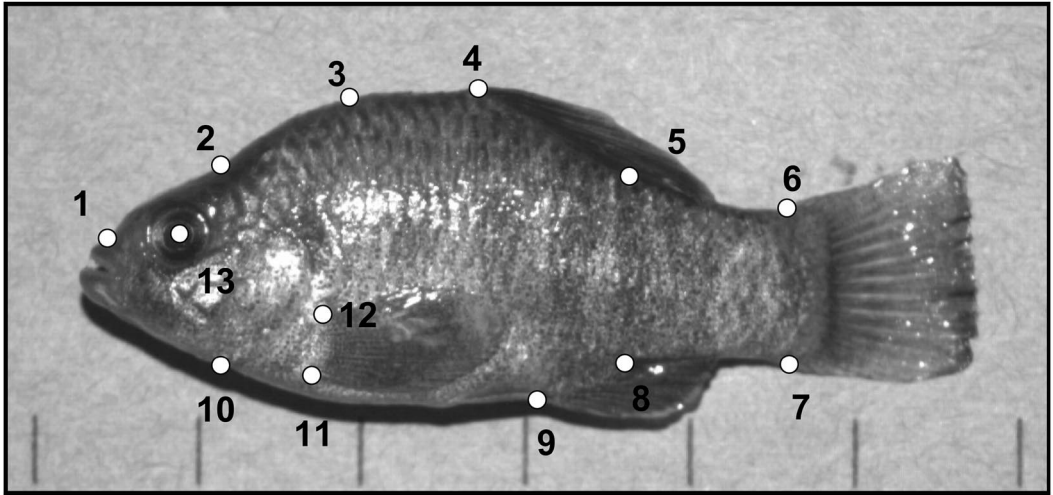


Fig. 1. Landmark definition for morphometric analyses of White Sands Pupfish. A male pupfish from Mound Spring is shown.

generate shape variables from x, y Cartesian coordinates of landmarks, with the effects of specimen size, orientation, and position held constant (Rohlf and Slice, 1990). All nonshape variation was held constant, mathematically, with a generalized Procrustes analysis (GPA) of original landmark data (Rohlf and Slice, 1990), which involves (1) a generalized least-squares superimposition of original landmark data scaled to the same size for all landmark configurations, and (2) centering subsequent size-free landmark configurations at the origin of a common coordinate system. The superimposed (aligned) landmark configurations represent points in a multidimensional shape space where relationships of specimens are defined by the metric, Procrustes distance (the square root of the sum of squared distances between homologous landmarks).

Differences in Procrustes distance (D_p) among population by sex groups (eight total) were assessed with permutation tests. Because we observed strong evidence for sexual dimorphism (see Results) individuals were randomly assigned to any of the four populations, within female and male blocks, for 4999 iterations. In each iteration, we calculated the test statistic, $|D_{pi} - D_{pj}|$, as a direct comparison of two shape differences, i and j . Direct comparisons were performed for females and males separately to compare shape divergence of Lost River and Mound Spring populations from Salt Creek to the shape differences between Salt Creek and Malpais Spring, the two native populations. The permutation tests produced distributions of 5000 statistics (including observed values) from

which the significance of observed values could be inferred as the probability of finding a greater distance by chance. Shape variation was graphically examined with the first three axes of a principal component analysis (PCA).

Although D_p provides an unscaled metric of shape difference between two points in shape space, the non-Euclidian nature of this space can be prohibitive for certain statistical analyses, such as the covariation of shape and other variables. The thin-plate spline (TPS) is a method that projects data from shape space into a tangent space that is Euclidian (Bookstein, 1989, 1991), thereby allowing statistical analyses of shape variation and covariation with other variables using linear models (Marcus, 1993; Marcus et al. 1996). We used TPS to generate shape variables (partial warp scores plus two uniform scores) from aligned landmark configurations produced by GPA (Bookstein, 1996; Rohlf and Bookstein, 2003). GPA and TPS were performed using the TPSRELW software, version 1.29 (F. J. Rohlf, 2003, unpubl.).

Analyses incorporating shape covariation with size.—To consider the covariation in shape and size (shape allometry), we used multivariate analysis of covariance (MANCOVA) for shape variables (Klingenberg, 1996). Independent variables included population, sex, size (covariate), and all interactions. The metric of size used in this analysis was centroid size (CS), which is calculated as the square root of the sum of squared distances of individual landmarks from the centroid of the landmark configuration (Bookstein, 1991). In this study, CS was highly correlated

with two other size measures, standard length (SL) and mass (Pearson $r = 0.99$ and 0.97 , respectively). The metric of shape difference between groups (accounting for shape allometry) was calculated as the magnitude of the shape vector between least-squares means. This value was converted to a generalized Mahalanobis (1936) distance, D_M , using the pooled within-group variance/covariance matrix of the multivariate model, to assess statistical significance.

Differences in shape allometry among population by sex groups were considered by comparing vectors of regression coefficients (\mathbf{b}), which describe the covariation of shape and size (see Appendix 1). The association of allometry vectors was considered by the angle, θ , between them, calculated from the equation: $\theta = \cos^{-1}(\mathbf{b}^T_i \mathbf{b}_j)$ for vectors i and j normalized to same unit length, where T represents a vector transpose. The vector correlation, r_V , is related to θ by the following equation: $r_V = \cos\theta$ (see e.g., Bégin and Roff, 2003). Thus, highly correlated vectors (i.e., that express similar covariation of shape and size) should have a small angle. Using a permutation procedure (described in Appendix 1), we generated distributions of 4999 random angles for every vector comparison. Vectors were considered significantly different if the angle between them was greater than or equal to the empirical angle associated with a one-tailed probability of $P_{rand} = 0.05$ from the random distribution of 5000 angles (including the observed value).

We also calculated θ between shape vectors between least-squares means to compare directional differences in shape, holding the effects of allometry constant. Shape vector comparisons included vectors between means of females and males (i.e., measures of sexual dimorphism), compared among populations, and vectors between SR and BS environments. Distributions of 5000 random angles were generated for each vector comparison with the same procedure for allometry vectors to determine the significance of vector directional differences.

Permutation procedures were performed using the software, Poptools, version 2.6.2 (G. M. Hood, 2004, unpubl.). We verified parameter estimates and multivariate test statistics for our multivariate model with the regression module of NTYSYS-pc, version 2.1 (F. J. Rohlf, 2002, Exeter Software, Setauket, New York). We also conducted a two-way ANOVA on CS for the independent variables, population and sex, to determine whether size differences existed among population by sex combinations, using the software, JMP, version 5.0.1 (SAS Institute, Inc., Cary, NC, 2002). Shape deformation grids were

produced from means of aligned coordinates, using the software, TPSSPLIN, version 1.20 (F. J. Rohlf, 2004, unpubl.), and for the regression of shape on size using the software, TPSREGR, version 1.28 (F. J. Rohlf, 2003, unpubl.).

RESULTS

Body shape variation.—For the shape differences we considered, Procrustes distances were largest between female and male means, within populations, and smallest between Salt Creek and Lost River means, suggesting that much shape variation could be attributed to sexual dimorphism (Table 1; Fig. 2). The only measure of sexual dimorphism that differed from others was that of Mound Spring, which was significantly less dimorphic ($P_{rand} < 0.0008$). Among-population patterns of shape variation were similar for both females and males, with the greatest distances occurring for comparisons of Mound Spring to SR populations. The divergence of Mound Spring shapes from Salt Creek was significantly greater than the divergence of Lost River shapes ($P_{rand} = 0.0002$ for both females and males) and was greater than the shape differences described for native populations ($P_{rand} = 0.0002$ for both females and males). Notably, the shape differences between Mound Spring and Malpais Spring were nearly as large as any other population comparisons, despite both populations occurring in springs.

Females had more slender body shapes than males and SR pupfish had more slender body shapes than BS pupfish (Fig. 2). Although BS pupfish were deep-bodied in both populations, the large difference in shape between them resulted from deep-bodied shapes produced by different landmark configurations. Deformation grids revealed that the snout (landmark 1) was more ventrally positioned, and the curvature of the dorsal crest (landmarks 2, 3, and 4) was more pronounced for Mound Spring females and males. In addition, the Mound Spring shapes exhibited narrower anal fins (landmarks 8 and 9) and shallower caudal regions (landmarks 5, 6, 7, and 8) for both females and males. Thus, although Salt Creek fish were introduced into an environment at Mound Spring that was more similar to Malpais Spring, body shape did not converge on the Malpais Spring form, despite becoming more deep-bodied.

Shape covariation with size.—MANCOVA indicated that the multivariate model for covariation of shape and size among population by sex groups was significant: (Wilks' $\Lambda = 0.00054$; Es-

TABLE 1. MATRICES OF BODY SHAPE DISTANCES FOR POPULATIONS OF WHITE SANDS PUPFISH. PROCRUSTES DISTANCES (D_p) ARE REPRESENTED IN ELEMENTS ABOVE MATRIX DIAGONALS AND GENERALIZED MAHALANOBIS DISTANCES (D_M) ARE REPRESENTED BELOW. DISTANCES CORRESPONDING TO MEASURES OF SEXUAL DIMORPHISM WITHIN POPULATIONS ARE ALSO SHOWN. ALL D_M VALUES ARE SIGNIFICANT ($P \ll 0.0001$). * DENOTES SIGNIFICANTLY SMALLER VALUE THAN D_p BETWEEN SC AND MO ($P_{rand} = 0.0002$). ** DENOTES SIGNIFICANTLY SMALLER D_p THAN OTHER MEASURES OF SEXUAL DIMORPHISM ($0.0002 < P_{rand} < 0.0008$). ABBREVIATIONS CORRESPOND TO POPULATIONS: LOST RIVER (LR), MALPAIS SPRING (MA), MOUND SPRING (MO), AND SALT CREEK (SC).

	Females				Males			
	LR	MA	MO	SC	LR	MA	MO	SC
LR	—	0.033	0.063	0.016* ^a	—	0.044	0.067	0.025* ^a
MA	4.219	—	0.059	0.024*	5.144	—	0.055	0.033*
MO	5.419	5.360	—	0.062	5.397	5.396	—	0.058
SC	2.639	3.334	4.861	—	2.983	4.465	4.582	—
Sexual Dimorphism		D_p		D_M				
LR		0.075		6.490				
MA		0.066		5.442				
MO		0.048**		4.545				
SC		0.071		5.230				

† These values were also significantly smaller than D_p between SC and MA.

timated $F_{330,4475} = 11.27$; $P < 0.0001$). All terms in the model were significant, including the Population \times Sex \times CS interaction (Wilks' $\Lambda = 0.763$; Estimated $F_{66,1064} = 1.53$; $P = 0.005$), indicating significant variation in shape allometry among population by sex groups. Further, CS significantly differed among population by sex groups ($F_{3,385} = 5.82$; $P = 0.0007$; Figs. 3, 4). Pairwise comparisons (using Tukey's honest significant difference tests) revealed that SR fish were significantly larger than BS fish and that females from Lost River were significantly larger

than males. Females and males were not different in size in the other three populations. However, Mound Spring fish were significantly smaller than Malpais Spring fish.

When accounting for the covariation of shape and size, all pairwise shape distances (D_M) considered were significant ($P < 0.0001$ for all distances; pairwise $\alpha = 0.0031$). Further, there was much concordance with D_M and D_p measures for all shape differences, with the exception that D_M between Mound Spring and Salt Creek was not larger than between Mound Spring and Malpais

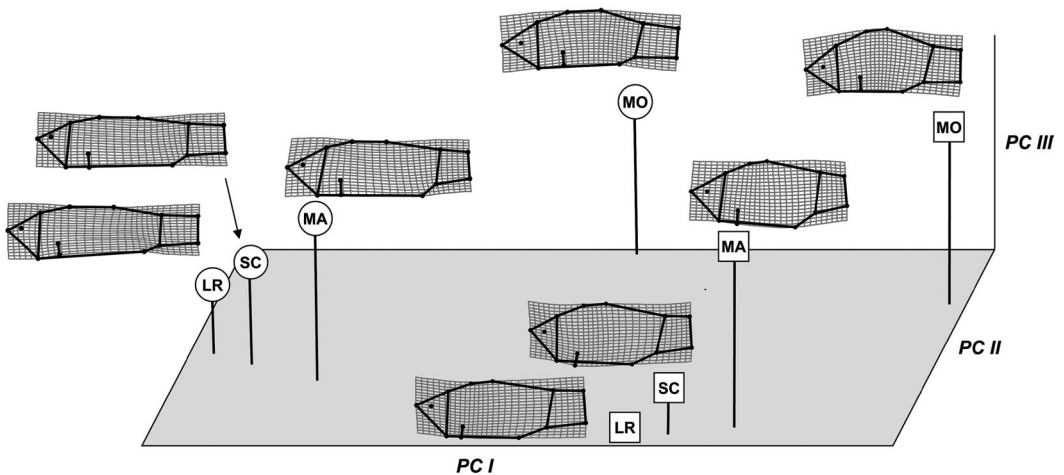


Fig. 2. First three principal components (PC) of shape variation for White Sands Pupfish. The three PCs shown represent approximately 69% of the total shape variation. Values in the plot correspond to group means for females (circles) and males (squares). Populations are indicated with the following abbreviations: Lost River (LR), Malpais Spring (MA), Mound Spring (MO), and Salt Creek (SC). Deformation grids (with a scale factor of $2\times$) are shown to facilitate a visual understanding of the shape corresponding to group means.

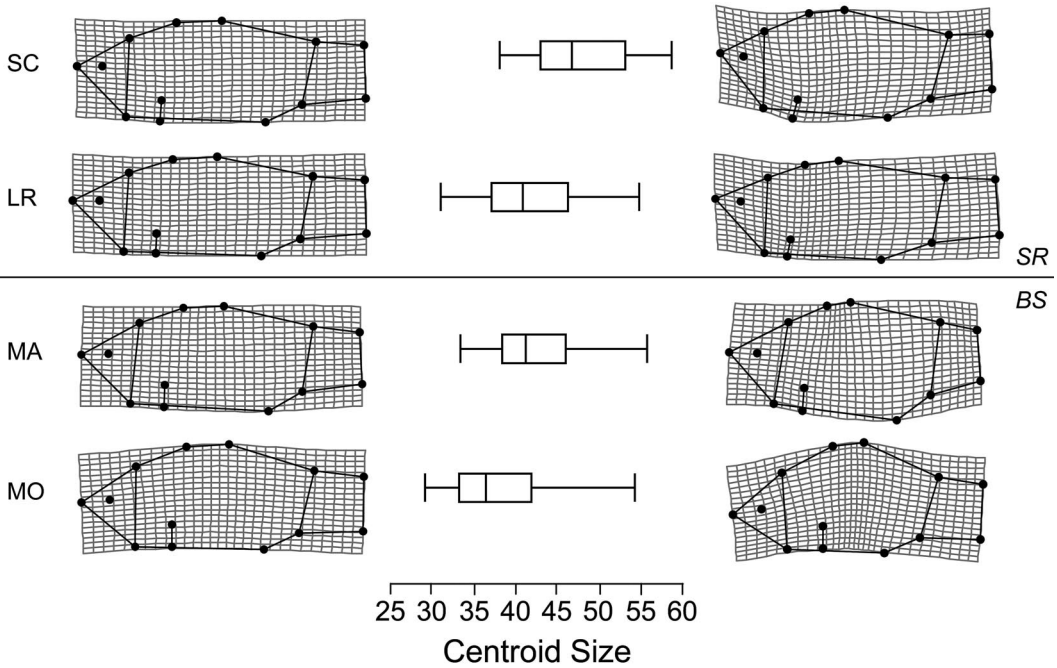


Fig. 3. Shape allometry of male White Sands Pupfish. Shape allometries are shown for each population (abbreviations the same as in Fig. 1) with saline river (SR) populations above and brackish spring (BS) populations below. Box plots show the range, 25th percentile, median, and 75th percentiles of centroid size data. Deformation grids are shown for the extents of the centroid size range to demonstrate the regression of shape on size. Deformation grids at large sizes are scaled 3× to facilitate a visual understanding of shape change associated with size.

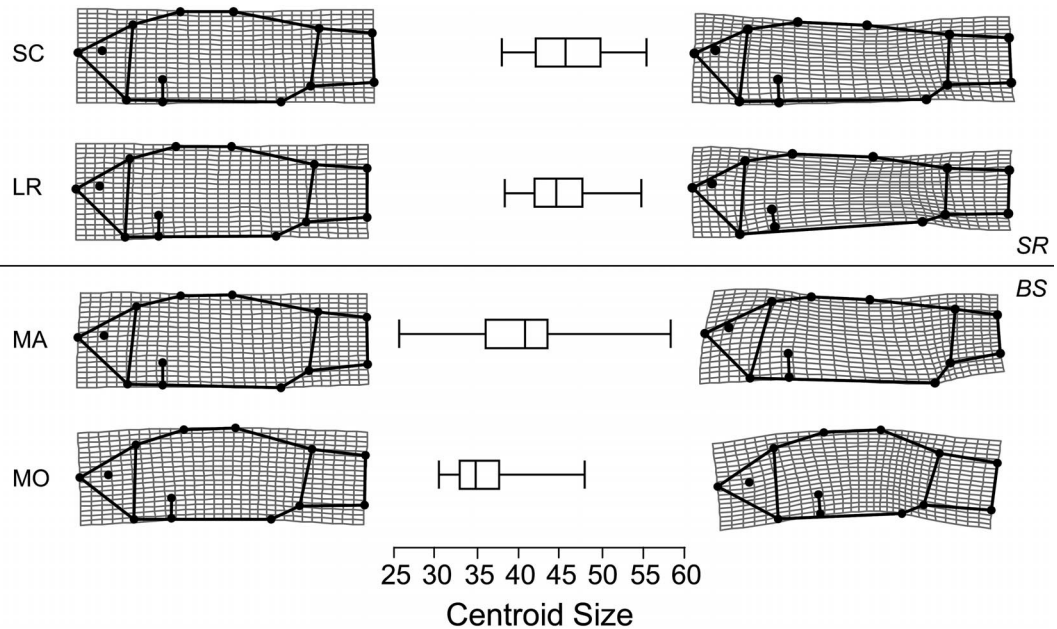


Fig. 4. Shape allometry of female White Sands Pupfish. Descriptions are the same as in Figure 3.

TABLE 2. MATRICES OF TEST STATISTICS FOR THE COVARIATION OF BODY SHAPE AND SIZE FOR POPULATIONS OF WHITE SANDS PUPFISH. Vector correlation coefficients, r_V , are represented in elements above matrix diagonals and angles, θ , (in degrees) are represented below. Test statistics for sexual dimorphism are also shown. Significantly different vectors ($r_V < 0.350$, $\theta > 69^\circ$) are italicized. Abbreviations are the same as in Table 1.

	Females				Males			
	LR	MA	MO	SC	LR	MA	MO	SC
LR	—	<i>-0.060</i>	<i>0.191</i>	<i>-0.043</i>	—	0.759	0.870	0.788
MA	<i>93.42</i>	—	0.376	<i>0.350</i>	40.61	—	0.688	0.616
MO	<i>78.97</i>	67.92	—	0.422	29.51	46.51	—	0.796
SC	<i>92.44</i>	<i>69.49</i>	65.01	—	38.04	51.96	37.21	—
Sexual Dimorphism		r_V	θ					
LR		0.454	63.015					
MA		0.600	53.159					
MO		0.373	68.113					
SC		0.482	61.183					

Spring for both females and males (Table 1). This finding does not alter the pattern of between-population differentiation in shape; although Mound Spring is slightly less divergent from Salt Creek when accounting for shape variation related to size variation (or shape and size covariation), either shape distance demonstrates that shape differences between Salt Creek and Mound Spring are greater than shape differences between Salt Creek and Malpais Spring.

The significant heterogeneity of shape allometries was caused by differences among populations within females (Figs. 3, 4; Table 2). Despite differences in shape among populations, shape allometries were not significantly different among populations for males. Each population showed a positive association with body depth, curvature of the dorsal crest, and size (Fig. 3). For Females, however, significantly different allometries were observed for all SR-BS comparisons except one (Salt Creek compared to Mound Spring; Fig. 4; Table 2). For Salt Creek and Lost River females, negative associations of body depth and size were observed, with Lost River fish having greater tapering of the posterior body. At larger sizes, Malpais Spring females showed a flattening of the dorsal crest and a more posterior position of the anal fin. Mound Spring females had exaggerated dorsal crests at larger sizes—a trend that shows some similarity to male shape allometries. Thus, the significantly smaller level of sexual dimorphism in shape for the Mound Spring population might be because of shape allometry that is more malelike for BS females compared to SR females (sexual dimorphism was also less for Malpais Spring than SR populations, but not significantly so; Table 1).

Directional differences in sexual dimorphism among populations were small but significant ($0.74 < r_V < 0.93$; $21.6^\circ < \theta < 41.2^\circ$; $0.0002 < P_{rand} < 0.031$). Differences were greatest for Mound Spring ($30^\circ < \theta < 41.2^\circ$). Directional differences for shape vectors between SR and BS populations were larger but not necessarily significant. The shape divergence of Mound Spring from Salt Creek was significantly different in direction ($P_{rand} = 0.002$ for both females and males) from Malpais Spring ($r_V = 0.48$ and 0.28 ; $\theta = 61.6^\circ$ and 73.9° ; females and males, respectively). However, the same pattern was not observed when Lost River was the SR population considered ($r_V = 0.44$ and 0.60 ; $\theta = 63.9^\circ$ and 53.5° ; $P_{rand} = 0.102$ and 0.333 ; females and males, respectively). Thus, although the difference in directions for shape vectors between SR and BS environments appear large compared to directional differences among sexual dimorphism vectors, these differences are not greater than expected by chance when comparing with Lost River. This result is not surprising given the larger shape distances observed for sexual dimorphism than interpopulation comparisons within sex.

DISCUSSION

Introduction of White Sands Pupfish from Salt Creek to new habitats lead to significant body shape divergence for new populations. Divergence in body shape was relatively modest for pupfish introduced to Lost River, a SR habitat like Salt Creek.

Both SR populations were characterized by body shapes that were more slender than BS pupfish. However, the divergence of body shape for pupfish in Mound Spring exceeded the level

of shape difference between Salt Creek and Malpais Spring, the two native populations that have been isolated since the desiccation of the Pleistocene Lake Otero (5000–10,000 years ago). The divergence of the Mound Spring body shape did not, however, converge on the Malpais Spring form, although both shapes were deep-bodied. This result indicates that deep-bodied shapes may be achieved by alternative landmark configurations and gives credence to the morphological distinctiveness of native populations.

Indeed, the Mound Spring body shape appears to have retained some features of a shape that evolved in a fluvial habitat. The shape of the head (more ventrally positioned snout) and shallow caudal region were consistent with the Salt Creek shape. Thus, it is possible that the significant shape difference between Mound Spring and Malpais Spring shapes is associated with genetic constraint (Oster and Albrecht, 1982; Bégin and Roff, 2003). This hypothesis is supported by the significant directional differences of SR-BS shape vectors (i.e., the shape vectors between Salt Creek and Malpais Spring and the shape vectors between Salt Creek and Mound Spring are uncorrelated). This result possibly indicates that the genetic covariances of Salt Creek and Malpais Spring populations differ; thus, shape response to similar selection differed between the two (see e.g., Klingenberg and Leamy, 2001; Bégin and Roff, 2003).

Overall, differences in shape allometry were subtle or not significant. Although the Mound Spring habitat may have altered the shape of pupfish introduced from Salt Creek, shape allometry did not significantly diverge. However, when compared to Lost River, the difference in shape allometry between the two introduced populations was significant for females. Mound Spring females had a positive association of curvature of the dorsal crest and size, much like males in all populations, but unlike females in Lost River. Thus, the level of sexual dimorphism was possibly significantly smaller for the Mound Spring population because mean shapes are, in part, a result of shape allometry. As a result, the female and male means of Mound Spring converge slightly in shape space. The greater sexual dimorphism of SR populations is best explained by the positive association of body depth and size for males but not for females. Nevertheless, the more slender body shapes of SR fish were distinct, regardless of the positive association of body depth and size for males. We outline two possible ecological explanations for such a relationship below.

First, body shape variation may be associated

with adaptive morphology related to water flow and salinity. The body shapes associated with Salt Creek and Lost River environments may reflect a combination of functional adaptations such as (1) an advantage to living in high water flow (Vogel, 1994), or (2) reduced circumference (compared to fish length) as a result of high osmotic potential in high salinity. The advantage of streamlined body shapes for fishes that live in flowing water, as an adaptation toward reduced energetic cost via drag resistance, is well documented (e.g., Blake, 1983; Weihs and Webb, 1983; Videler, 1993). Low-speed maneuverability and neutral buoyancy can be augmented by highly compressed, laterally flattened body shapes that facilitate pivoting motions (Webb, 1997), which may be less energetically costly in the spring environment. Therefore, shape variation between SR and BS environments may reflect greater streamlining of body shape in the SR environments. However, a tendency for pupfish to have slender body shapes in other, perhaps less fluvial, saline environments has been noted (e.g., Miller, 1948). Experimental research on White Sands Pupfish native populations has indicated that body shape is, in part, phenotypically plastic with a negative association of body depth and salinity (Collyer, 2003). Therefore, difference in body shape between SR and BS pupfish may indicate a phenotypically plastic response to variation in salinity levels among habitats. Such a hypothesis is supported by the observed Lost River body shapes. Lost River Pupfish (which were introduced from the Salt Creek population) were slightly more slender than Salt Creek shapes and the Lost River habitat is slightly more saline (Stockwell and Mulvey, 1998).

Second, body shape variation may indicate sexual difference in behavior. Previous studies (e.g., Cowles, 1934; Raney et al., 1953; Echelle, 1973) have documented breeding territory defense of males in *Cyprinodon*. Barlow (1961) documented lateral threat displays with *Cyprinodon macularius* that were also observed with *Cyprinodon pecosensis* by Kodric-Brown (1977, 1978), who also noted that visual cues were important in territorial defense by male *Cyprinodon* against conspecific males. Breeding territory defense by males has also been documented for the White Sands Pupfish (Suminski, 1977). Male size is an important determinant in *Cyprinodon* for the ability of males to obtain and defend territories (Kodric-Brown, 1978). Thus, deep-bodied shapes may be associated with increased fitness because of a functional or display advantage of deep-bodied males to defend territories. Further, this hypothesis is supported by the de-

creased association of body depth and size for females, who do not defend breeding territories.

The results of this study warrant further investigation into the adaptive significance of body shape in White Sands Pupfish and the concomitant management of native populations. Based on the genetic distinctiveness of native populations (Stockwell et al., 1998) and their geographic isolation in dissimilar environments, the native populations of White Sands Pupfish are managed as separate evolutionarily significant units (ESU; Ryder, 1986; Waples, 1991; Stockwell et al., 1998). Consistent with management strategies of short-lived desert fish species (Hendrickson and Brooks, 1991), the establishment of refuge populations, as a hedge against extinction of native populations, is a goal of the current management plan. Because Lost River and Mound Spring populations could potentially serve as refuge populations of the native Salt Creek population, it is important to evaluate how novel environments could affect the evolutionary legacy of the Salt Creek ESU in refuge environments. Our results indicate that contemporary morphological evolution of refuge populations is a potential concern.

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morph), written and maintained by F. J. Rohlf. The Poptools software is available at <http://www.cse.csiro.au/poptools>, written and maintained by G. M. Hood.

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APPENDIX 1

STATISTICAL DETAILS

The multivariate linear model used to describe shape vector relationships was of the form $\mathbf{Y} = \mathbf{XB} + \boldsymbol{\epsilon}$, where \mathbf{Y} is the matrix of partial warp scores and uniform scores, \mathbf{X} is the design matrix coded for the effects of interests (e.g., sex, population, and size), \mathbf{B} is a matrix of regression coefficients, and $\boldsymbol{\epsilon}$ is a matrix of residuals. From the multivariate model, vectors of (population by sex) least-squares means, \mathbf{y} , were calculated. A shape vector, $\Delta\mathbf{y}$, describes a difference in two group vectors (e.g. between female and male

means for one population, or between population means within females or males). The magnitude of shape difference between two groups (adjusting for effects in the model) is the Euclidian distance of the shape vector: $D_i = \|\Delta\mathbf{y}_i\| = (\Delta\mathbf{y}_i^T \Delta\mathbf{y}_i)^{1/2}$ for comparison, i .

We compared the directions of different vectors by calculating the angle between them:

$$\theta = \cos^{-1}\left(\frac{\Delta\mathbf{y}_i^T \Delta\mathbf{y}_j}{D_i D_j}\right),$$

for shape vectors i and j (Bégin and Roff, 2003). This is the same procedure for calculating the angle between allometry vectors, \mathbf{b} , which are vectors of regression coefficients from the matrix \mathbf{B} , pertaining to size covariates described in \mathbf{X} .

To test the significance of θ for either shape vector or allometry vector comparisons, a permutation procedure was performed. Because significant population and sex effects were observed in MANCOVA, we used a procedure that preserved these effects. First, we determined the parameter estimates from the model $\mathbf{Y} = \mathbf{X}_1\mathbf{B}_1 + \boldsymbol{\epsilon}_1$, where \mathbf{X}_1 contained effects coded for population and sex. For each permutation procedure iteration, the residuals were randomly permuted twice, to form two matrices of residuals, $\boldsymbol{\epsilon}_{1,1}^*$ and $\boldsymbol{\epsilon}_{1,2}^*$, where the superscript, *, represents a randomized form of the matrix. These matrices were then used to calculate \mathbf{Y}_1^* and \mathbf{Y}_2^* , holding \mathbf{B}_1 constant. The randomly generated response matrices were used in the model $\mathbf{Y}^*_i = \mathbf{X}_2\mathbf{B}_2 + \boldsymbol{\epsilon}_2$, where \mathbf{X}_2 contained effects and size covariates coded for population by sex groups. Random shape vectors and allometry vectors were calculated from the two sets of data, as well as θ between corresponding vectors (i.e., θ was calculated as the angle between two random versions of the same vector).

We performed 4999 permutation iterations to generate distributions of random angles, holding population and sex effects constant. This allowed us to test the null hypothesis (no difference in direction between observed vectors) by calculating the probability that distributions of randomly generated angles contained observed angles. For every vector comparison, we calculated $P_{rand} = P(\theta_{random} > \theta_{observed})$ to infer the significance of directional differences in vectors. Two assessments were performed for every comparison: $\theta_{observed}$ was calculated for two vectors; and θ_{random} was measured for one vector (i.e., $\theta_{observed}$ was compared to two distributions of θ_{random}). Any observed angle was not considered significant if we failed to reject the null hypothesis (at $P_{rand} < 0.05$) for either random distribution.