

Variability of morphology–performance relationships under acute exposure to different temperatures in 3 strains of zebrafish

Christina L. Miller^{a,b,*}, Robert Dugand^c, and Katrina McGuigan^a

^aSchool of the Environment, The University of Queensland, Brisbane, QLD 4072, Australia

^bDepartment of Plant Biology, Michigan State University, MI 48823, USA

^cSchool of Biological Sciences, The University of Western Australia, Crawley, WA 6009, Australia

*Address correspondence to Christina L. Miller. E-mail: christina.miller@uqconnect.edu.au

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Abstract

Locomotion is thermally sensitive in ectotherms and therefore it is typically expressed differently among thermally heterogeneous environments. Locomotion is a complex function, and whereas physiological and behavioral traits that influence locomotor performance may respond to thermal variation throughout life, other contributing traits, like body shape, may have more restricted responses. How morphology affects locomotor performance under variable temperature conditions is unknown. Here, we investigated 3 genetically distinct strains of zebrafish, *Danio rerio* (AB, WIK, and Tu) with a shared multi-generational history at 28 °C. After rearing fish at 28 °C, we measured prolonged swimming speed (U_{crit}) at each of 6 temperatures (between 16 °C and 34 °C). Speed was strongly positively correlated among temperatures, resulting in most among individual variation being temperature-independent (i.e., fish were relatively fast or slow across all temperatures). However, we also detected significant variation along 2 axes reflecting temperature-dependent variation. Although strains differed in mean swimming performance, within strain (among-individual) patterns of speed variation were markedly consistent. Body shape and size explained significant variation among individuals in both temperature-independent and temperature-dependent axes of swimming speed variation. Notably, morphological traits that were most strongly associated with temperature-independent performance variation (i.e., faster–slower) differed from those associated with temperature-dependent (i.e., hotter–colder) variation. Further, there were significant differences among strains in both the direction and strength of association for specific morphological traits. Our results suggest that thermally heterogeneous environments could have complex effects on the evolution of traits that contribute to whole organism performance traits.

Key words: *Danio rerio*, multiple regression, swimming speed, thermal performance, trait correlation, U_{crit} .

Motile organisms rely on locomotion to undertake diverse activities, including finding food and mates. The repeated evolution of specific environment–locomotor performance relationships provides strong evidence for local adaptation of locomotor performance (e.g., McGuigan et al. 2003; Ghalambor et al. 2004; Langerhans et al. 2004; Hendry et al. 2011; Dalziel et al. 2012). An individual's locomotor performance can also vary plastically in response to environmental factors. For example, speed typically varies with ambient temperature in ectotherms (e.g., Condon et al. 2010; Latimer et al. 2014; Logan et al. 2018). Temperature-dependent performance, characterized by thermal performance curves (Huey and Kingsolver 1989), is interpreted with respect to the thermal sensitivity of enzymatic reactions underpinning physiological traits (Hochachka and Somero 2002; Angilletta et al. 2003). Temperature variation can occur over different spatial and temporal scales, including diurnal and seasonal changes, with physiological processes and locomotor performance similarly exhibiting rapid (< 1 h) and more gradual (seasonal acclimation) responses to these changes in ambient temperature (Schulte et al. 2011; Seebacher et al. 2014).

Plastic responses to temperature, and their persistence (i.e., whether plasticity is irreversible or reversible) can not only depend on the life-stage of the organism and the duration of exposure but can also be trait-dependent (Kellermann et al. 2019; Salinas et al. 2019; Pottier et al. 2022). In contrast to physiological and behavioral traits, morphological traits typically exhibit irreversible plasticity, being sensitive to temperature during development (e.g., Elphick and Shine 1998; Frazier et al. 2008; Sfakianakis et al. 2011), but, once development and growth are completed, morphological traits lose the capacity to respond to subsequent changes in temperature. Yet locomotor performance depends on morphological as well as physiological (and behavioral) traits. For example, fish body shape and size affect swimming speed via influences on drag and stability, whereas speed further depends on physiological traits, such as the contractile properties of muscle fibers (Videler 1993). Morphological trait values and swimming speed are correlated among individuals (e.g., Langerhans and Makowicz 2009; Hendry et al. 2011; Conradsen and McGuigan 2015) and undergo correlated evolution under divergent selection regimes

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(e.g., [McGuigan et al. 2003](#); [Dalziel et al. 2012](#); [Kern et al. 2016](#)). If, and how, the contribution of physiology, morphology, and behavior to locomotor performance changes with ambient temperature has received relatively little attention.

Drosophila melanogaster reared at colder temperatures develop larger wings and have better flight performance than warm-reared flies from the same population ([Frazier et al. 2008](#)), suggesting coordinated developmental plasticity of morphology and performance. In contrast, although both morphology and locomotor speed responded to rearing temperature in the lizard *Bassiana duperreyi*, the change in morphology did not explain the change in speed ([Elphick and Shine 1998](#)). Notably, [Kolok \(1992\)](#) found that different traits explained variation in swimming speed among individual large-mouth bass (*Micropterus salmoides*) in summer versus winter, suggesting that the causal basis of inter-individual variation in locomotor performance might be seasonally dependent. Such heterogeneity in performance correlations might be important for adaptive evolution in natural populations. If selection acts on other traits through their contribution to locomotor performance ([Arnold 1983](#); [Walker 2007](#)), then changes in the magnitude or sign of the correlation of those traits with locomotor speed will cause heterogeneity in selection on these causal traits, even when selection on the emergent function, locomotor speed, remains consistent.

Here, we investigate the relationship between morphology and swimming speed in zebrafish, *Danio rerio*. We specifically sought to determine whether body shape or size could explain the variation in the response of swimming speed to acute changes in temperature. Fish were reared to adulthood under constant thermal conditions of 28 °C, and their swimming speed measured during acute (< 4 h) exposure to 6 test temperatures: 16, 20, 24, 28, 31, and 34 °C. We characterized the variation among individuals in their swimming speed across this temperature range and determined whether morphology explained the observed inter-individual variation. We further determined how the relationships between static morphology and thermally dependent performance varied among 3 genetically differentiated populations (strains). Overall, our results suggest that morphology explains substantial variation in both temperature-independent and temperature-dependent swimming performance and that these relationships are genetically variable.

Materials and Methods

Study system

We assayed the swimming speed and morphology of 3 widely studied zebrafish strains: AB, WIK, and Tu. These strains have independent histories and are genetically differentiated ([Holden and Brown 2018](#); [Suurväli et al. 2020](#)), consistent with being collected from independent wild populations. Local populations of each strains were established 3 to 5 generations prior to this experiment by fish imported from the Zebrafish International Resource Center. For the current experiment, clutches were collected from 5 breeding groups (each consisting of 4 males and 4 females) per strain and reared in 3.5 L tanks on a recirculating water system (for details on husbandry see [Conradsen et al. 2016](#)). Under these common-environment rearing conditions, phenotypic differences among strains can be attributed to genetic differences, whereas variation within strains will reflect both genetic and

environmental (among and within rearing tank) effects, which cannot be partitioned under this breeding design.

After ~90 days postfertilization (dpf), sexually mature males were tagged using an elastomer tag (Northwest Marine Technology, Inc.) following protocols described in [Conradsen and McGuigan \(2015\)](#). These individually identifiable males were assigned to groups of 6, with at least 1 fish per strain in each group, and were maintained in their group for the duration of the experiment. We had aimed to also assess a population founded by crossing AB × Tu, and fish from this population were included in each group of 6 fish. However, due to poor breeding success, exacerbated by data loss due to equipment failure (detailed below), sample size for this strain was very low, insufficient for robust statistical analysis. These data were thus removed prior to any analyses.

Swimming performance

We assayed the swimming speed of fish at each of six temperatures: 16 °C, 20 °C, 24 °C, 28 °C, 31 °C, and 34 °C. Zebrafish are a diurnal, shallow water, schooling fish with a broad altitudinal and latitudinal distribution across the Indian subcontinent. They are found across a wide thermal range (16 °C to 34 °C) and experience acute daily changes in temperature ([Spence et al. 2006](#); [Engeszer et al. 2007](#); [Arunachalam et al. 2013](#)). In lab culture, zebrafish are maintained at a constant temperature; our cultures are maintained at 28 °C, similar to the conditions experienced by their ancestor at the Zebrafish International Resource Center (28.5 °C; [Westerfield 2007](#)). In the current study, experimental temperatures were chosen to include the rearing temperature of 28 °C, and to span the species-wide range of average temperatures, while excluding more extreme temperatures that represent stressful conditions at which locomotion is inhibited ([Condon et al. 2010](#)). Performance typically declines from the optimum gradually toward colder temperatures, but precipitously toward hotter temperatures ([Izem and Kingsolver 2005](#)). This, in combination with the fact that zebrafish rearing temperature, chosen to ensure rapid growth and high reproductive rates, is closer to the upper than lower thermal limits ([Lawrence 2007](#)), led us to use a slightly smaller interval (3 °C) between the 2 assay temperatures above 28 °C than between the 3 assay temperatures below 28 °C (4 °C interval).

Swimming trials followed a blocking design, where different groups encountered temperatures in different orders to ensure temperature was not confounding with age or experience, or time of day (diurnal effects). Water in the flume was maintained at temperatures 28 °C and above using an aquarium tank heater (1500 W Titan Heavy Duty Aquarium Heater, Quian Hu, Singapore) and at temperatures 24 °C and below using an aquarium tank chiller (440 W TECO Tank Chiller, Ravena, Italy). Prior to swimming, fish were placed in tanks that were gradually cooled (at a rate of 0.20 °C per minute) or warmed (0.27 °C per minute) from 28 °C to the test temperature.

Swimming speed was characterized as critical swimming speed, U_{crit} , using a step-velocity test ([Brett 1964](#)) in a flume with a swim chamber of 46 cm × 14 cm × 13 cm (L×W×H) (30 L capacity, Loligo systems, Tjele, Denmark). Two flumes were initially used, but one malfunctioned, returning unreliable data. Data from this flume were discarded, and these assays were not repeated in the other flume, resulting in some loss of data (final sample sizes are detailed below). The step-velocity protocols are detailed in [Conradsen and McGuigan \(2015\)](#). Briefly, as soon as the swim group

reached the assay temperature, the fish were introduced to the flume, and allowed to settle at low flow (4 cm^{-1}) for 15 min. The flow rate was then increased by 4 cm^{-1} every 5 min (300 s). Each of the 6 individual fish were removed when they could no longer hold station, whereas the remaining fish continued at the test speed. The U_{crit} of each fish was calculated as $U_{\text{crit}} = U_i + (U \times (T_i/T))$ (Brett 1964), where U_i was the maximum velocity (cm^{-1}) maintained for the full step interval (T ; here, 300 s), U was the water speed increment (here, 4 cm^{-1}), and T_i was the time (in seconds) the individual fish swam at their final velocity step. By providing individuals of this schooling species the opportunity to swim in a group, U_{crit} estimates might be inflated because of stress mitigation or kinematic benefits of schooling (e.g., Killen et al. 2012; Burgerhout et al. 2013). Heterogeneity in such benefits across temperatures or among strains may contribute to unexplained (residual) variation in U_{crit} .

U_{crit} was assayed for a total of 148 individuals (47, 48, and 53 of AB, Tu, and WIK respectively), with 118 individuals measured across at least 4 temperatures. Data were missing at random with respect to temperature and strain, with a minimum (maximum) of 29 (40) individuals assayed per strain per temperature. Due to the missing data, we did not investigate multivariate outliers here. One (of 600) U_{crit} observation was extreme (>3 standard deviation (SD) above mean) and strongly inflated among-individual variance at that temperature; we therefore excluded this observation from all analyses.

Morphology

Fish were anesthetized (AQUI-S, Lower Hutt, New Zealand) and photographed on their left side using a tripod mounted Nikon Coolpix camera. A 1 mm grid was included in all photographs. Images were randomly ordered, and the position of 12 landmarks was recorded using TPSdig2 (Rohlf 2005; Figure 1). Fish size was recorded as standard length (SL), the distance from the anterior tip of the snout (LM1) to the origin of the caudal fin (LM6). Landmark positions were then aligned using generalized Procrustes fit, implemented in MorphoJ (Klingenberg 2008, 2011). The shape was characterized by 10 Inter-Landmark Distances (ILDs, Figure 1) following Conradsen and McGuigan (2015) in units of centroid size, calculated as the Euclidean distance between aligned landmark co-ordinates using R (RStudio Core Team 2019). Each fish was photographed 3 times: during the first (150–157 dpf), after the third (233–240 dpf) and after the final (257–264 dpf) set of swim trials. We analyzed the average

(over the 3 repeated measures) trait values per individual. On average, fish grew 0.5 mm (where average SL was 28.1 mm) between the first and second measures, and 0.04 mm between the second and third. One individual was identified as a multivariate outlier based on Mahalanobis distance ($\alpha = 0.001$, $df = 11$) (Mahalanobis function in base R, RStudio Core Team 2019) and excluded from morphological analyses.

Data analyses

Divergence among strains in thermal performance and morphology

There are several approaches for analyzing thermal performance data (Izem and Kingsolver 2005; Gomulkiewicz et al. 2018). Our experiment, where individuals were assayed across a fixed set of 6, relatively evenly separated temperatures that were well within the thermal limits of the species, are well suited to a multivariate approach (Latimer et al. 2014; Gomulkiewicz et al. 2018). This approach treats the swimming performance phenotype as a set of ordered values (i.e., speed at each temperature: Gomulkiewicz et al. 2018), where we aim to first test the null hypothesis that the mean performance among strains does not vary among the 6 temperatures. To do so, maximum likelihood, implemented in PROC MIXED in SAS (SAS Institute Inc. 2011), was used to fit the model:

$$y_{ijklmno} = \mu + S_i + T_j + ST_{ij} + B_k + D_l + G_m + I_{n(im)} + \varepsilon_{ijklmno} \quad (1)$$

where y was the vector of U_{crit} measures ($o = 1 \dots 599$), and μ was the overall mean U_{crit} . The effect of the i^{th} ($i = 1 \dots 3$) strain (S) and j^{th} ($j = 1 \dots 6$) temperature (T), along with their interaction (ST_{ij}) were fit as categorical fixed effects. The effect of the k^{th} ($k = 1 \dots 6$) swimming block (B) and the l^{th} ($l = 1, 2$) diurnal period (D, morning or afternoon) were also modeled as categorical fixed effects. Block (where fish were randomly allocated to a different temperature in each of the $k = 1 \dots 6$ repeated swimming blocks) captured variation due to age and experience, as well as potential temporal trends in general conditions. The effect of the m^{th} ($m = 1 \dots 29$) swim group (G) was modeled as a random effect to account for variation among mixed-strain groups of fish that were housed together and swam together. The effect of the n^{th} ($n = 1 \dots 148$) individual (I), nested within strain, was also fit as a random effect, where the covariance among temperatures (repeated measures of the same individual at multiple temperatures), was constrained to be positive definite. The residual

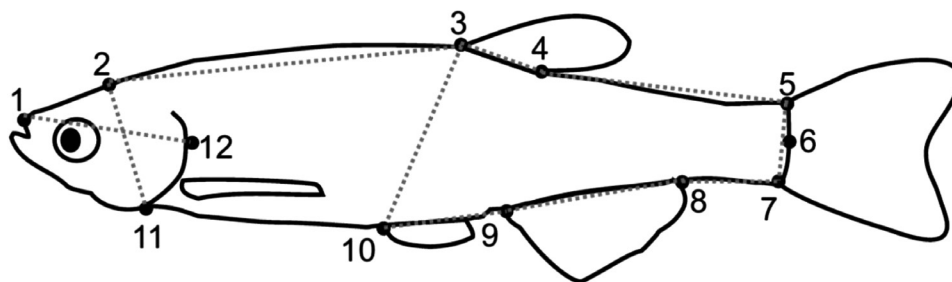


Figure 1. Schematic of *D. rerio* indicating the position of the 12 landmarks (black dots) and 10 inter-landmark distances (ILDs, gray dotted lines) used in this study. Landmarks were: anterior tip of the snout at the upper jaw (1); nape (2); dorsal-fin origin (3); dorsal-fin insertion (4); dorsal insertion of caudal fin (5); median caudal fin insertion (6); ventral insertion of caudal fin (7); ray of the anal fin (8); anal-fin origin (9); pelvic-fin origin (10); ventral posterior point of the operculum (11); posterior most point of the operculum (12). The 10 ILDs are referred to by their landmark endpoints (e.g., ILD1-12 is the distance between landmarks 1 and 12).

Table 1. Dimensionality of among-individual thermal performance. The fit (AIC; -2LL), number of estimated parameters, and results of the likelihood ratio tests are reported for models with none through 4 dimensions (i.e., eigenvalues > zero) of among-individual variation in swimming speed across the 6 temperatures. The difference in fit (Δ -2LL) between a model and the next dimension model (none to 1; 1 to 2, etc.) follows a chi-square distribution with degrees of freedom equal to the difference in number of parameters

Dimensions	AIC	-2LL ¹	Parameters	Δ -2LL	df	P-value
None	4044.3	4040.31	2			
1	3931.1	3917.09	8	123.22	6	<0.0001
2	3919.7	3895.73	13	21.36	5	0.0007
3	3909.1	3879.10	17	16.62	4	0.0023
4	3913.3	3875.35	20	3.76	3	0.2891

¹-2 log likelihood ratio.

(ε) captured unexplained variation among the repeated measures per individual.

We applied log-likelihood ratio tests (LRT), comparing the fit (-2 log-likelihood) when the term of interest (the strain-by-temperature interaction or strain) was modeled versus when it was not; this difference in fit follows a chi-square distribution with the degrees of freedom (df) equal to the number of parameters differing between the 2 models (here, 1) (Liang and Self 1996). The LRT is robust to errors in calculating the appropriate df , given the complex error structure (highly correlated repeated measures and unequal sample sizes per individual and strain); conclusions were identical between this approach and an F -ratio test. Conclusions were also identical when assumptions about the rank of among-individual variance, homogeneity of among-individual variance among strains, and homogeneity of the residual were relaxed.

Next, we assessed whether strains differed in multivariate morphology. PROC GLM in SAS (SAS Institute Inc. 2011) was used to fit the model:

$$y_{ij} = \mu + S_i + \varepsilon_{ij} \quad (2)$$

where y was a vector of the 11 morphological traits per individual, μ was the vector of global means per trait, and strain (S) was modeled as a categorical fixed effect. Individual, nested within strain was the random residual error, ε . We retained the normalized linear discriminant functions from this analysis and calculated individual scores along each of the 2 discriminant functions for plotting. Inclusion of size as a response variable allowed us to succinctly determine whether strains were morphologically divergent, considering both size-dependent (allometric) and independent shape changes. However, these traits differ in scale (SL in cm versus $ILDs$ in units of centroid size) which could influence conclusions. We applied model (2) to data on the raw measurement scale, and to variance standardized data (mean = 0, SD = 1 for each trait). Conclusions (strains were morphologically distinct, with WIK and Tu differing the most) were consistent across both analyses, and we present only results on the variance standardized scale.

Performance: morphology relationships

An advantage of the multivariate approach to analyzing thermal performance data is that it can be coupled with eigenanalysis to define new traits (the eigenvectors) that characterize temperature-independent or temperature-independent variation (Izem and Kingsolver 2005; Latimer et al. 2014). Thus, we first estimated the 6×6 matrix of the variance

among individuals in their speed at each of the 6 temperatures, and their covariance of speed between each pair of temperatures, and then derived the eigenvectors from this matrix to define performance traits for further analysis.

The among-individual matrix was estimated using restricted maximum likelihood (REML) to fit a modified form of model (1) containing only random effects. Data were centered (mean = 0) on their respective level of each of the categorical fixed effects prior to analysis, which is equivalent to fitting these in the model, but improves efficiency; these mean-centered data were retained to calculate performance scores (detailed below). There was little evidence that strains differed in among-individual variation (Supplementary Materials; Table S1 and S2), and we therefore used all data to define the new performance traits.

Consistent with individual speed being correlated across temperatures, 3 performance traits (i.e., the eigenvectors; Table 1) were statistically supported by factor analytic modeling (Meyer and Kirkpatrick 2005; Hine and Blows 2006) (see Supplementary Materials). To calculate individual scores on these 3 performance axes (using the mean-centered data), we first excluded individuals with fewer than 4 U_{crit} observations, retaining 118 individuals ($AB = 40$, $Tu = 40$, and $WIK = 38$). For fish missing 1 or 2 U_{crit} observations, the missing data were replaced with the mean swimming speed deviation of that strain at the missing temperature(s). These data were then multiplied by their respective loadings on the eigenvectors of among-individual variation in U_{crit} .

For each independent performance axis, we first tested the hypothesis that the 3 strains shared the same morphology-performance relationship. Strains differed (see section Results) in both their U_{crit} (and hence scores on the performance axes) and morphology. To investigate the relationship between morphology and performance separately from these differences, we first centered and scaled (i.e., calculated z -scores) the data within each strain. We fit 2 linear regression models (using the `lm` function in R RStudio Core Team 2019): one with the 11 morphological traits as continuous predictors, and a second in which strain-by-morphological trait interactions were also fit. These models were compared via LRT (implemented in the `lmer` package, Zeileis and Hothorn 2002). Where the null hypothesis of among-strain homogeneity of slopes was rejected, the `emmeans` package (Lenth 2024) was used to further investigate differences.

Given evidence of strain-specific slopes (see section Results), we analyzed data within each strain to determine the best model to explain thermal performance variation for each

strain. Using the “regsubsets” function in the “leaps” package in R (RStudio Core Team 2019), an exhaustive search was made of all possible additive linear models, including from one up to all 11 morphological trait predictors. The 10 best-fit (based on residual sums of squares) models were retained for each model size (i.e., number of predictors, with only one 11-trait model; 101 models were retained per performance trait and strain). We identified the best-fit model based on Mallows’ Cp criterion. To support robust interpretation of differences among strains, we also report the model averaged regression coefficients from a subset of models within 2 Akaike Information Criterion (AIC) of this best-fit model (implemented using the R package “MuMIn” Bartoń 2024).

Results

Divergence among strains

Strains differed in how their mean swimming speed changed with temperature (i.e., their thermal performance: strain-by-temperature interaction, $X^2 = 29.98$, $df = 1$, $P < 0.0001$; Figure 2). This significant interaction is likely contributed both by temperature-dependent changes in the relative magnitude as well as the direction of differences among strains in their mean speed. In particular, inspection of the means (Figure 2) suggests that although WIK had the slowest mean speed at all temperatures, the magnitude of the difference was not constant. Tu was markedly faster than either WIK or AB at the coldest temperatures, whereas AB was fastest at the hottest temperatures (although AB and Tu had similar speeds at these temperatures) (Figure 2).

Strains also differed significantly in their morphology (Wilk’s $\lambda = 0.09$, $F_{22, 260} = 28.01$, $P < 0.0001$). WIK and Tu were the most strongly differentiated, with the higher mean among-species linear discriminant function 1 (DFS_1) score of Tu (Figure 3) reflecting their longer (SL), shallower bodies

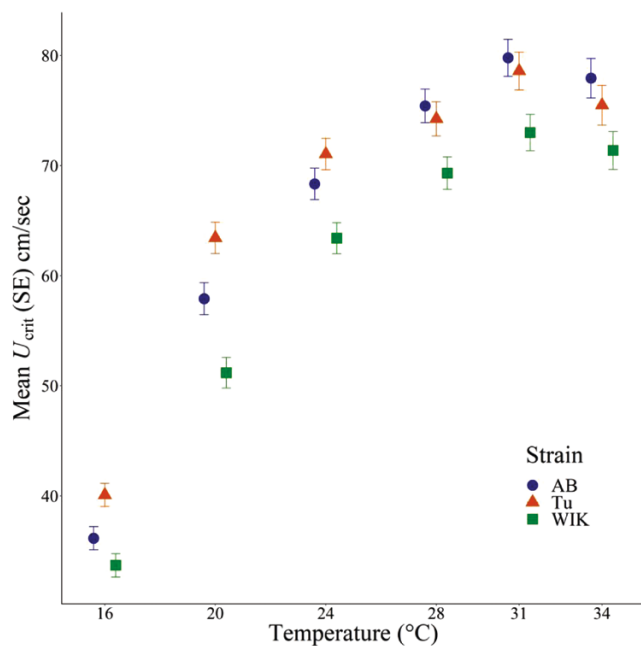


Figure 2. Swimming speed variation of 3 zebrafish strains across 6 temperatures. The least-square means (\pm SE) from model (1) are plotted for each strain. The points are offset to allow a visualization of each strain at each temperature.

(ILD 3.10) (DFS_1 ; Table 2). AB was differentiated from the other strains along the second discriminant axis (Fig. 3), reflecting a relatively short dorsal caudal peduncle (ILD 4.5) (DFS_2 ; Table 2).

Variation among individuals in swimming performance

All temperatures contributed to the major axis of among-individual variation in swimming performance in the same direction, with relatively similar magnitude of loadings (with the weakest and strongest contribution from 16 °C and 31 °C, respectively, reflecting heterogeneity in the magnitude of among-individual variances at each temperature: diagonals of Supplementary Table S1) (e_1 ; Table 3). This pattern reflects the pervasive positive cross-temperature correlations of speed (off-diagonal elements of Supplementary Table S1) and suggests that most among-individual variation in speed was independent of temperature (i.e., a faster–slower axis of variation).

The second axis of among-individual variation in performance was characterized by contrasting contributions from speed at cool (20 °C and 24 °C) and warm (31 °C) temperatures (e_2 ; Table 3), somewhat consistent with a hotter–colder pattern of variation: individuals that were relatively fast at colder temperatures were relatively slow at hot temperatures (particularly 31 °C) (and vice versa). The third axis was dominated by speed at 34 °C (e_3 ; Table 3), which contributed little to the other 2 axis of variation. Speed at 34 °C was relatively weakly correlated with speed at other temperatures (Supplementary Table S1), and thus e_3 captures the independent determination of speed at this, hottest, temperature.

Morphology–performance relationships

For e_1 , which predominantly reflected temperature-independent (faster–slower) variation in speed, morphology explained significant variation in all 3 strains (Table 4), but the morphology–performance relationships were strain-specific (LRT of model with versus without strain-specific slopes: $X^2 = 42.69$, $df = 22$, $P = 0.0052$; Supplementary Table S3). Caudal peduncle depth (ILD5–7) had the greatest difference in effect, strongly positively associated with higher e_1 scores (i.e., faster swimming) in Tu, but with a weak negative effect on speed in AB and no effect in WIK (Table 5, Supplementary Table S4). Speed increased with greater distance between pelvic- and anal-fin origins (ILD9–10) in both Tu and AB, but this trait again did not influence e_1 scores in WIK (Table 5, Supplementary Table S4). Longer heads (ILD1–12) were strongly associated with slower swimming in Tu, but weakly implicated as increasing speed in the other strains (Table 5, Supplementary Table S4). In WIK, speed increased with SL , but was independent of length in the other 2 strains (Table 5, Supplementary Table S4).

There was also evidence that morphology explained significant variation along the second performance axis, e_2 , in each strain (Table 4), but that strains differed in how morphology influenced this performance (LRT: $X^2 = 37.25$, $df = 22$, $P = 0.0222$). This overall support for strain-specific morphology–performance relationships reflected relatively weak differences in slope of individual traits (Supplementary Table S3). Specifically, Tu fish with high e_2 scores (i.e., fish that swam relatively fast at 31 °C but relatively slowly at 20 °C and 24 °C) had relatively anteriorly positioned dorsal fins (i.e., short ILD2–3), but this trait had a weakly opposing

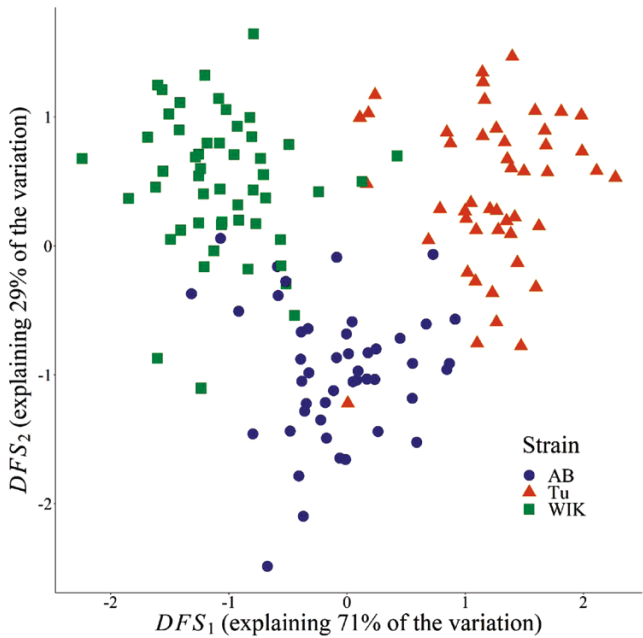


Figure 3. Variation in morphology among strains. Individual scores along the 2 discriminant functions are plotted by strain. The percent of among-strain variation is shown on the respective axis.

Table 2. Discriminant functions of morphological variation among strains. The proportion of among-strain variance (%) accounted for, and normalized trait loadings are presented for the 2 among-strain linear discriminant functions (DFS_1 and DFS_2). Traits are defined in Figure 1

	DFS_1	DFS_2
	71.0%	29.0%
SL	0.648	−0.067
ILD1-12	−0.041	−0.004
ILD2-11	0.367	−0.033
ILD2-3	−0.162	0.484
ILD3-4	−0.027	0.421
ILD3-10	−0.478	−0.298
ILD4-5	−0.082	0.544
ILD5-7	0.168	−0.249
ILD7-8	−0.194	0.269
ILD8-9	−0.340	0.245
ILD9-10	0.018	−0.055

effect on e_2 performance in WIK and no effect in AB (Table 5, Supplementary Table S5). In WIK, fish with shallow caudal peduncles (ILD5-7) had high e_2 scores, whereas in AB there was a weakly positive slope, and no effect in Tu (Table 5, Supplementary Table S5).

For e_3 (predominantly reflecting variation in speed at 34 °C), there was no evidence that strains differed in how morphology influenced scores ($X^2 = 21.50$, $df = 22$, $P = 0.4902$), but morphology did influence e_3 scores (pooled analysis of all strains: $F_{1,115} = 4.14$, $P = 0.0443$, adjusted $R^2 = 0.026$). Only one trait was included in the best-fit model—higher e_3 scores (i.e., faster speed at 34 °C) were associated with shorter heads (ILD1-12: $\beta = -0.186$, $SE = 0.092$). These results suggest that, although individuals varied significantly along this performance axis (Supplementary Table S2), very little of that

Table 3. Among-individual variation in swimming performance. The covariance matrix of among-individual variation in U_{crit} at the 6 assay temperatures (for all strains pooled, constrained to 3 dimensions; Supplementary Table S1) was subject to eigenanalysis. The eigenvalues (top row; as a proportion of total variation in second row) and normalized eigenvector loadings for each temperature are reported

	e_1	e_2	e_3
	135.63	31.08	19.37
	72.9%	16.7%	10.4%
16 °C	0.138	−0.267	−0.021
20 °C	0.439	−0.436	−0.087
24 °C	0.392	−0.463	−0.074
28 °C	0.431	−0.111	−0.103
31 °C	0.563	0.689	−0.384
34 °C	0.363	0.193	0.910

variation could be explained by morphological variation. Notably, although there was no statistical support for heterogeneity of slopes between strains, morphology explained significant variation along e_3 in only Tu (Table 4). Tu fish with higher e_3 scores had shorter heads (consistent with the pooled model) and longer posterior dorsal lengths (ILD3-4 and ILD4-5; Table 5).

Discussion

Locomotor performance is complex, influenced by morphological, behavioral, and physiological traits that are themselves determined by genes and environment. Temperature is well known exert immediate effects on locomotor performance via effects on the contractile properties of skeletal muscle (Angilletta et al. 2002; James and Tallis 2019). Temperature can also elicit plastic changes in behavior, which may either compensate for or exacerbate the potential fitness consequences of temperature effects on performance (reviewed in James and Tallis 2019). How morphology, which does not respond to acute temperature changes, contributes to variation in performance under thermally variable conditions remains relatively unexplored. Examining variation in body shape and in prolonged swimming speed across a temperature gradient, we found that external morphology explained patterns of both temperature-independent and temperature-dependent performance. The morphological trait associations were specific both to the axis of thermal performance variation and to the genotype (strain).

Given the broad literature reporting correlated evolution (e.g., Langerhans 2009; Kern et al. 2016; Cano-Barbacid et al. 2020) or within population covariation (e.g., Hendry et al. 2011; Conradsen et al. 2016) of morphology and speed measured at a single temperature, we expected body shape to predict temperature-independent speed. Consistent with this, morphology explained variation among individuals on the major axis of performance variation, e_1 , an axis that most closely resembled a faster–slower (Izem and Kingsolver 2005) mode of thermal performance. In contrast, we expected that thermally dependent performance variation would be due to variations in musculature and enzymes independent of the external morphological traits we analyzed. However, body shape also explained significant variation among individuals along e_2 , a performance axis that reflected a hotter–colder (Izem and Kingsolver 2005) pattern where individuals that

were relatively fast at cooler temperatures (20 °C and 24 °C) tended to be relatively slow at hotter temperatures (31 °C) and vice versa.

Within each strain, the different axes of swimming performance variation were typically associated with different morphological traits, with little evidence that the same trait had either concordant or antagonistic effects on temperature-independent (e_1) and temperature-dependent (e_2) axes of variation. Indeed, when considering the 3 strains and 2 major axes of swimming performance, all 11 morphological traits were represented in at least one best-fit model, yet only ventral inter-fin length (ILD9-10) in Tu was associated with higher performance scores on both axes, whereas in WIK dorsal caudal length (ILD4-5) had weakly antagonistic effects on the 2 performance axes (Table 5). Below, we discuss 3 broad factors that are important for interpreting these results.

First, we note that the mechanisms that may connect static morphology to dynamic performance are not clearly

established. The viscosity of water decreases with increasing temperature, which may affect swimming independently of the physiological effects of temperature (Fuiman and Batty 1997; von Herbing and Keating 2003; Danos and Lauder 2012). Although viscosity has the greatest impact on small (larval) fishes (Fuiman and Batty 1997; von Herbing and Keating 2003; Yavno and Holzman 2018), complex effects on larger fishes (including adult zebrafish) have been documented (Danos and Lauder 2012). Temperature-dependent physiological rates and viscosity can both influence tail beat frequency and amplitude (Fuiman and Batty 1997; Danos and Lauder 2012), which may change recoil dynamics during swimming. Notably, although strains differed in their morphology- e_2 associations (Table 5, Supplementary Table S5), in general, implicated traits capture changes in the positioning of dorsal and paired fins, which may influence recoil (yaw) (Webb 2006; Borazjani 2013; Conradsen and McGuigan 2015). Numerical simulations have extended our

Table 4. Regression of swimming performance on morphology. Results for the regression model with the lowest Mallow's C_p are reported for each strain and performance axis (regression coefficients for included morphological variables are reported in Table 4). We note that, because performance scores were calculated on mean-centered data, the R^2 values are expected to be upwardly biased, akin to the known inflation of heritability estimates in mixed models including fixed effects (Wilson 2008)

e_1	<i>F</i>	df	<i>P</i>	Adj R^2
AB	3.62	4,35	0.0144	0.212
Tu	11.81	4,35	<0.0001	0.526
WIK	10.41	2,34	0.0003	0.343
e_2				
AB	3.89	3,36	0.0166	0.182
Tu	5.99	3,36	0.0020	0.277
WIK	3.29	3,33	0.0169	0.196
e_3				
AB	1.19	1,38	0.1748	0.023
Tu	4.98	3,36	0.0055	0.234
WIK	1.01	1,35	0.3239	<0.001

Table 5. Regression coefficients from the best-fit model of swimming performance on morphology. For each axis of performance variation (e_1 , e_2 , e_3), the standardized beta coefficients, β (\pm SE), are reported for strain-specific models (note: for e_3 only the results for Tu are reported here). Morphological traits are defined in Figure 1; performance traits are defined in Table 4. Where the null hypothesis of zero slope was rejected ($\alpha = 0.05$) values are shown in bold

	e_1			e_2			e_3
	AB	Tu	WIK	AB	Tu	WIK	Tu
SL			0.551 (0.135)				
ILD1-12		-0.566 (0.148)					-0.286 (0.166)
ILD2-11		-0.297 (0.156)		0.368 (0.149)		0.316 (0.179)	
ILD2-3					-0.330 (0.147)		
ILD3-4				-0.400 (0.154)			0.501 (0.184)
ILD3-10	0.423 (0.271)						
ILD4-5	0.566 (0.212)		0.242 (0.135)			-0.290 (0.165)	0.486 (0.201)
ILD5-7	-0.334 (0.168)	0.512 (0.121)				-0.428 (0.168)	
ILD8-9				0.240 (0.150)	-0.273 (0.144)		
ILD9-10	0.346 (0.186)	0.286 (0.116)			0.385 (0.139)		

understanding of the mechanisms of swimming (Tokić and Yue 2012; Gazzola et al. 2014). Simulations exploring fine-scale (intra-specific) variation, coupled with further empirical experiments, could provide novel insights into the contribution of external morphology to locomotor performance, and how this might vary with environmental effects. Ideally, further empirical investigations would also characterize the physiological traits expected to contribute to temperature-dependent performance to determine how they co-vary with morphology.

Second, we emphasize that although regression tests causal hypotheses (i.e., body shape causes performance values) it does not provide evidence of causation. Manipulative experiments can be effective at confirming causative effects—for example, experimental fin clips have supported the causal role of fins in determining swimming speed (e.g., Wakamatsu et al. 2019). However, such experiments are impossible or impractical for most traits. Replicated natural co-evolution of trait and performance (e.g., Langerhans 2008) are suggestive of a causal effect, but may arise from responses to independent selection pressures. Correlated evolution of performance in response to artificial selection on a putatively causal trait (e.g., Kern et al. 2016) may provide a more compelling inference. Such an approach has not yet been applied to the question of how body shape influences thermally dependent swimming speed.

Finally, morphological covariance with independent axes of thermal performance variation could influence interpretations of adaptive evolution. Many studies have demonstrated broadly consistent patterns of phenotypic evolution among locomotor niches (e.g., Langerhans 2008, 2009), suggesting a common morphology–performance relationship. However, these studies also typically reveal unique responses in different populations inhabiting broadly similar environments (Langerhans 2018; Heckley et al. 2022). Including thermal variation in habitat descriptions, alongside well-studied factors such as water flow, structural complexity, and predation, may improve our understanding of locomotor performance evolution.

Studies of traits influencing locomotor performance predominantly consider taxa (populations or species) with divergent trait values. To predict future evolutionary responses, we need to better understand the factors determining within population variation. Our observation of consistent ranking of fast versus slow individuals, irrespective of temperature, (i.e., along e_1) suggests that performance capacity is repeatable; repeatability sets an upper limit on the evolutionary potential of a trait (Boake 1989; Wilson 2018). Previous studies, at a single temperature, have demonstrated that locomotor performance is moderately repeatable over various timescales (reviewed in Conradsen et al. 2016). However, repeatability (or heritability) of temperature-dependent locomotor performance has received less attention (Careau et al. 2014; Latimer et al. 2014; Logan et al. 2018; Miller et al. 2023). Our observation that e_2 , reflecting temperature-dependent (hotter–colder) variation in U_{crit} , was associated with a similar variation in the 3, genetically divergent, strains (Supplementary Table S2) suggests that it also captures repeatable differences among individuals. In contrast, the statistical support for strain-specific morphological predictors of performance suggests that the morphology–performance map may be more variable than the performance itself.

Notably, strains differed in the contribution of caudal peduncle depth (ILD5-7) to among-individual differences in

speed. The caudal region plays a key role in determining swimming performance, with hydrodynamic principals (Walker 1997; Blake 2004) and empirical studies (e.g., Langerhans et al. 2004; Langerhans 2008) revealing that larger caudal regions generate greater thrust but increased drag, increasing the maximum speed, but decreasing endurance. Conradsen et al. (2016) observed strong, positive slopes of caudal peduncle depth on U_{crit} at 28 °C in repeated measures (~3 months apart) of both male and female WIK zebrafish. Here, WIK caudal peduncle depth was not associated with overall speed (e_1 scores), although fish with relatively deep caudal peduncles had low e_2 scores (i.e., fish that were relatively fast at 20 °C and 24 °C but slow at 31 °C had deeper caudal peduncles: Table 5). The morphology–performance map of Tu for e_1 (Supplementary Tables S4) closely matched that of the most similar-age WIK fish in the previous study (normalized vector dot product = 0.79, where 1.0 would indicate concordance and 0.0 orthogonal vectors) (Supplementary Table S4 vs. Assay 1 in Table 2 of Conradsen et al. 2016). Thus, our results provided equivocal evidence that morphological traits had repeatable associations with the repeatable axes of locomotor performance.

In conclusion, our study has provided some evidence that static morphology may contribute to variation in thermally dependent swimming speed. We suggest that both modeling approaches and incorporation of data on thermal regime into studies of natural divergence in locomotor performance may improve our understanding of causal effects on locomotor capacity. We also highlight the paucity of data on within-population variation in locomotion and its putatively causative traits, and the consequently limited knowledge of what proportion of observed phenotypic variation reflects heritable genetic differences among individuals. Ongoing statistical developments and increasing accessibility of genomic data may provide opportunities to investigate the genetic covariance of performance and morphological traits in natural populations.

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Conflict of Interest Statement

The authors declare no conflicts of interest.

Data Availability

Data available UQ eSpace: <https://doi.org/10.48610/2a7ddb6>.

Supplementary Material

Supplementary material can be found at <https://academic.oup.com/cz>.

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