

Genotype and temperature affect locomotor performance in a tiger salamander hybrid swarm

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Summary

1. Anthropogenic environmental changes have the capacity to disrupt natural population dynamics. For amphibians with complex life cycles, it is important to understand how environmental perturbations interact with variation in larval period duration to affect the timing of dispersal and the abiotic conditions under which terrestrial movements occur.

2. Widespread hybridization between California tiger salamanders and introduced tiger salamanders has created a situation in which length of larval development and metamorphic timing varies because of extrinsic variation in abiotic factors such as temperature, and also as a result of individual-specific variation in genomic admixture.

3. We examined how line-cross type (e.g. F1, F2, backcrosses, etc.), morphology and temperature interact to affect the performance of emigrating immature salamanders. We performed endurance trials on a mechanized treadmill to simulate dispersal following metamorphosis. Our study provides insights into the interaction of environmental change and genomic composition on locomotor performance and the spread of the hybrid swarm.

4. We found that temperature had a significant positive impact on endurance, potentially making it an important factor driving dispersal distances in the wild. Line-cross type also affected performance, with F1 hybrids demonstrating the greatest movement capacity. However, we did not observe an interaction between line-cross type and temperature.

5. These results demonstrate that an increase in ambient temperature may enhance the dispersal ability of hybrid individuals and accelerate the spread of the hybrid swarm. An increase in the rate of introgression of non-native tiger salamander alleles into formerly pure native habitats places enhanced importance on the proper management of populations near the boundary of the hybrid swarm and emphasizes the need for management actions that prevent the intentional or accidental translocation of non-native species.

Key-words: *Ambystoma tigrinum*, *Ambystoma californiense*, climate, dispersal, hybrid vigour, hybridization, migration

Introduction

Amphibians with a biphasic life cycle face a basic ecological tradeoff. Prolonged larval periods can lead to larger sizes at metamorphosis, increased survival in the post-metamorphic terrestrial phase, a shorter time to sexual maturity and overall greater fitness (Semlitsch, Scott & Pechmann 1988; Scott 1994; Morey & Reznick 2001). However, long larval periods can also lead to increased risk of desiccation-associated larval mortality as vernal pools dry, and post-metamorphic mortality if metamorphosis occurs during hot, dry conditions when overland movements are difficult or impossible.

Interacting with these life history tradeoffs are the landscapes and climatic conditions where species and populations have evolved. For example, the southeastern US tends to have abundant summer rainstorms that promote successful terrestrial migrations of vulnerable young metamorphs, whereas most of California has a Mediterranean climate with virtually none of the summer rains that aid dispersal elsewhere. Predictably, the solutions to these life history tradeoffs faced by many metamorphosing amphibians vary geographically and taxonomically in keeping with local rainfall and climate patterns.

In response to variable pond hydroperiods and rainfall patterns, closely related species of amphibians may display profound variation in the degree to which metamorphic

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Fig. 1. Left: Adult hybrid tiger salamander (*Ambystoma tigrinum* × *californiense*) from Monterey County, California; Right: Adult California tiger salamander (*A. californiense*) from Solano County, California; photo by J. R. Johnson.

timing is a plastic or relatively fixed life history feature. Within our tiger salamander study system, species display tremendous life history variation in metamorphic timing. The barred tiger salamander (BTS; *Ambystoma tigrinum mavortium*; Fig. 1) has the ability to coordinate metamorphosis and subsequent emigration with biotic factors (e.g. prey abundance, competitor density) and aspects of the abiotic environment (e.g. pond hydroperiod, temperature, humidity, rainfall) to optimize fitness (Wilbur & Collins 1973). As the BTS is native to the US Great Plains and breeds in both semi-permanent and fishless permanent ponds (Petranka 1998), plasticity in larval period often leads to a delay in metamorphosis and exploitation of aquatic habitats with little risk of catastrophic early pond drying. Summer rains contribute additional yearly variation in the hydroperiods of native BTS breeding habitats and provide a moist terrestrial environment for emigration of newly metamorphosed juveniles periodically throughout the summer and fall.

In contrast, the California tiger salamander (CTS; *Ambystoma californiense*; Fig. 1) exhibits remarkably little plasticity in the timing of metamorphosis. CTS evolved in the Mediterranean climate of California's Central Valley where natural aquatic habitats (shallow vernal pools) have consistently short hydroperiods and rainfall is essentially absent following the winter breeding season (Trenham *et al.* 2000). Peak metamorphosis occurs from late June to early July (Shaffer & Trenham 2005) as ponds dry, at which time CTS juveniles must make nighttime, overland movements to upland retreats (usually rodent burrows or soil cracks) without the benefit of rainfall (Loredo, Vanvuren & Morrison 1996). The choice of burrow may be extremely important to emigrating salamanders because drift-fence studies suggest that the initially chosen retreat is continuously occupied for the next several months (Searcy & Shaffer 2008). Individuals that fail to find shelter will perish if exposed to daytime temperatures and dryness (Loredo, Vanvuren & Morrison 1996), and desiccated juveniles are periodically found on the surface (C. Searcy & B. Shaffer, unpublished data). Interestingly,

even when pond hydroperiods are unusually long, CTS tend to metamorphose at small sizes and forego the potential for increased larval growth and larger metamorphic size both in the laboratory (J. Johnson, B. Fitzpatrick & B. Shaffer unpublished data) and in the field (C. Searcy & B. Shaffer, unpublished data). In natural California landscapes, the benefits of plasticity in metamorphic timing during rare wet years apparently do not outweigh the importance of early metamorphosis or the costs associated with the maintenance of phenotypic plasticity (Relyea 2002).

Hybridization between CTS and BTS has resulted from the intentional release of BTS from the southwestern US in the 1950s into the Salinas Valley (Riley *et al.* 2003; Fig. 2). Previous studies have found both hybrid vigour (Fitzpatrick & Shaffer 2007a) and hybrid dysfunction (Fitzpatrick 2008) in the larval stage and have demonstrated that larval CTS are at a competitive disadvantage in experimental mesocosms (Ryan, Johnson & Fitzpatrick 2009). While this hybrid system has been well studied with respect to the geographical patterns of introgression and larval fitness (Fitzpatrick & Shaffer 2004, 2007a, b; Fitzpatrick *et al.* 2009, 2010), the consequences of admixture have not been investigated for any post-metamorphic fitness components in the lab or the field.

It is well established that the novel genetic variation produced through hybridization generates phenotypic variation in the timing of metamorphosis that greatly exceeds that found in native CTS populations under laboratory and mesocosm conditions (Ryan, Johnson & Fitzpatrick 2009, J.R. Johnson, B.M. Fitzpatrick & H.B. Shaffer unpublished). What remain unclear are the consequences of increased plasticity and longer larval period for post-metamorphic salamanders. One possibility is that delayed metamorphosis may

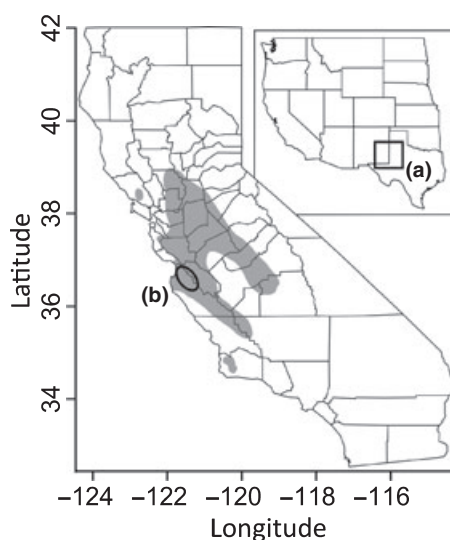


Fig. 2. Range of California tiger salamanders outlined by grey shading in California's Central Valley. The inset displays the Western United States and the black box denoted by (a) represents the likely source of the introduced barred tiger salamanders from New Mexico and Texas. The oval denoted by (b) represents the main extent of the hybrid swarm in the Salinas Valley, Monterey County, California.

negatively affect the fitness of late-metamorphosing juvenile salamanders given the extreme environmental conditions experienced during late emigration, when daytime temperatures can exceed 40 °C and rain is non-existent. However, elevated ambient temperatures have been shown to improve the locomotor performance of ectotherms (Bennett 1990) including tiger salamanders (Else & Bennett 1987), and extreme daytime highs may have a negligible effect on salamander fitness because movements occur at night when temperatures are consistently much cooler. Therefore, hybrids that delay metamorphosis might experience an increase in survival if increased locomotor capacity translates into a greater chance of finding an appropriate underground refuge during predawn field conditions.

In this study, we examined locomotor performance of both pure and early-generation hybrid juvenile salamanders to identify differences that could impact fitness based on line-cross type (e.g. F1, F2, backcrosses, etc.) or phenotype (e.g. mass). Variation that is attributable to line-cross type is by definition genetically controlled and provides insights into the genetic basis of differences among species. We replicated our experiments at two temperatures to explore the physiology of temperature-dependent locomotor capacity and potential genotype-by-temperature interactions. Our goal was to determine the contributions of temperature and size on locomotor performance of recently metamorphosed salamanders to shed light on how environmental and morphological variation might impact fitness of recently metamorphosed tiger salamanders in the wild. Importantly, an improved understanding of locomotor capacity will help predict the dynamics and spread of the hybrid swarm that currently threatens the remaining populations of pure CTS (Fitzpatrick & Shaffer 2007a,b). Given the endangered status of the California Tiger Salamander and the importance of hybridization as a key threat to the species (US Fish and Wildlife Service 2004), understanding the fitness consequences of hybridization is also a critical element of endangered species management in this system.

Materials and methods

STUDY ANIMALS

J.R. Johnson, B.M. Fitzpatrick & H.B. Shaffer (unpublished) generated 1st and 2nd generation hybrids [i.e. F1, F2, and backcrosses (bcCTS & bcBTS)] between *Ambystoma californiense* and *A. tigrinum mavortium*, as well as control parental crosses for both species. Larvae were reared in individual containers from hatching through metamorphosis and a random selection of metamorphs was maintained for this study. Line-cross sample sizes were: CTS ($N = 6$), BTS ($N = 5$), bcCTS ($N = 10$), bcBTS ($N = 9$), F1 ($N = 5$) and F2 ($N = 6$). Following metamorphosis, salamanders were maintained individually in the laboratory at 20 °C on a 12 h light to 12 h dark photoperiod prior to experiments.

Salamanders from each cross type were run on a treadmill at each of two different temperatures and were randomly assigned to one of two regimes – hot first or cold first – to account for any potential effect of trial order. All animals were fed 3 days before initiation of trials

and all members of a trial regime (hot or cold first) were kept for 3 days at either 15 °C or 25 °C. These trial temperatures were chosen to represent the average field temperatures observed during times of juvenile salamander dispersal as measured via iButtons (Maxim Integrated Products, Sunnyvale, CA, USA) set to record the temperature every 30 min *in situ* at a nearby breeding site and averaged over 3 years (see Fig. S1 in Supporting Information). Average nighttime ambient temperatures during late June–early July (time period of peak metamorphosis; Shaffer & Trenham 2005) are about 15–20 °C (Fig. S1), although evaporative cooling may produce lower salamander body temperatures than those recorded by the iButtons.

PERFORMANCE TRIALS

Following acclimation, salamanders were selected at random and were run individually to exhaustion on a mechanized treadmill. The treadmill was constructed within a 65 cm long \times 15.3 cm wide \times 22 cm deep clear plastic frame (Fig. S2). The treadmill speed was set to match the empirically determined maximum speed of each individual independently. To accomplish this, at the beginning of each trial, the treadmill speed was adjusted to match the apparent maximum initial walking speed of each individual salamander. This speed varied for individual animals, but remained constant during that individual's trial. As salamanders became tired, they were maintained in constant maximum forward motion by tapping or lightly pinching the tail if needed. Tests for fatigue were performed every 3 min, or when salamanders refused to walk any further. Fatigue was determined by a righting response test – the inability of a salamander to right itself physically within 3 s after being placed on its back (Shaffer, Austin & Huey 1991; Austin & Shaffer 1992). Each trial was terminated at fatigue and each animal was tested only once at each temperature. After a trial was terminated, walking speed, total walking duration and mass were recorded. Distance travelled was calculated as the product of speed and duration walked.

DATA ANALYSES

Data analyses were conducted in R (R Core Development Team, 2009). The effects of temperature, line-cross type and mass were evaluated using maximum likelihood estimation of linear mixed-model regression with the R package 'nlme.' We used temperature, line-cross type, and mass as fixed effects and individual ID as a random effect in each model. Measures of performance (i.e. distance walked and speed walked) were log-transformed ($\ln[x + 1]$) to improve data normality. We standardized mass across line-cross types by using the difference between each individual's mass and its line-cross type mean value to remove the dramatic differences in mass among line-cross types (Fig. S3). The use of standardized values allows us to investigate the effect of mass on performance independent of line-cross type. We also parameterized models using untransformed and log-transformed mass, but do not report those results because the same best-fit models were selected in each case. We performed an analysis of variance on the best-fit models and evaluated line-cross-type contrasts with native CTS values as a reference. We were primarily interested in evaluating the hypothesis that hybrid cross types performed differently from native CTS individuals and hence we did not perform all pairwise contrasts among line-cross types.

For 'speed walked', we calculated Q_{10} (for rate-based responses) values, and for 'distance walked', we calculated R_{10} (for quantity-based responses) values, which represent the change in locomotor performance over a ten-degree change in temperature: $Q_{10} = [R2/R1]$,

Table 1. Means and standard deviations for each locomotor performance variable

Cross type	N	Mass (g)		Distance (m)				Speed (cm s ⁻¹)				Q ₁₀	
				15 °C		25 °C		15 °C		25 °C			
		Mean	SD	Mean	SD	Mean	SD	R ₁₀	Mean	SD	Mean		SD
CTS	6	10.8	2.8	39.0	8.9	1082.1	1098.4	27.7	11.5	1.3	18.1	3.5	1.6
bcCTS	10	11.3	2.8	95.8	59.3	1507.4	1080.4	15.7	12.3	2.4	21.1	7.5	1.7
F1	5	13.7	2.7	261.8	124.7	1778.2	931.1	6.8	12.7	2.6	21.3	5.7	1.7
F2	6	15.3	6.7	50.7	20.3	647.1	809.5	12.7	11.2	1.6	12.7	3.2	1.1
bcBTS	9	20.7	7.7	148.0	115.3	1234.8	726.1	8.3	11.8	2.2	16.9	4.8	1.4
BTS	5	26.5	9.9	160.8	190.8	682.2	543.5	4.2	14.8	3.2	18.3	7.4	1.2

where R is the rate of the response variable at temperatures 1 and 2, and $R_{10} = [S_2/S_1]$, where S is the value of the response variable at temperatures 1 and 2 (Bennett 1984). The temperature coefficient (Q_{10}) and the thermal ratio (R_{10}) are analogous terms that are used to describe the thermal dependence of the response variables and allow qualitative comparison among studies investigating thermal responses.

Results

Sample sizes, mean mass and performance trial results are presented in Table 1. As found in more formal quantitative genetic analyses, CTS are much smaller than BTS, and hybrid categories are intermediate following a roughly additive model (J.R. Johnson, B.M. Fitzpatrick & H.B. Shaffer unpublished). We observed a dramatic increase in locomotor performance of all line-cross types at 25 °C relative to 15 °C. Mean duration of trials (averaged across line-cross types) at 15 °C was 17 min vs. 102 min at 25 °C. This difference in trial duration translates into a difference of more than 1 km travelled or a 30% increase in walking speed on average (Fig. 3).

Model selection revealed that while temperature had the greatest effect on performance, line-cross type and mass were also important (Table 2). Overall, the best-fit model for distance walked included temperature, line-cross type, mass and the interaction between line-cross type and mass. The best-fit model for speed walked included additive effects of temperature and line-cross type only (Table 2). The best three-factor model for speed walked was not a significant improvement over the model including only temperature and line-cross type (Table 2, Table S1). We found no evidence of a significant interaction between temperature and line-cross type, suggesting that all cross types displayed a similar pattern of improved performance from cold to hot trials. Qualitatively, however, CTS demonstrated a dramatic increase in distance walked at higher temperatures and BTS showed the least response, with hybrid cross types in the middle of these two extremes (Table 1, Fig. S4). For speed walked, we observed a positive response to increasing temperatures for the smallest cross types (bcCTS, CTS, and F1), while the larger categories maintained more constant speeds across the temperature treatments (Table 1). For all response variables, temperature had the largest effect, followed by additive effects of line-cross type.

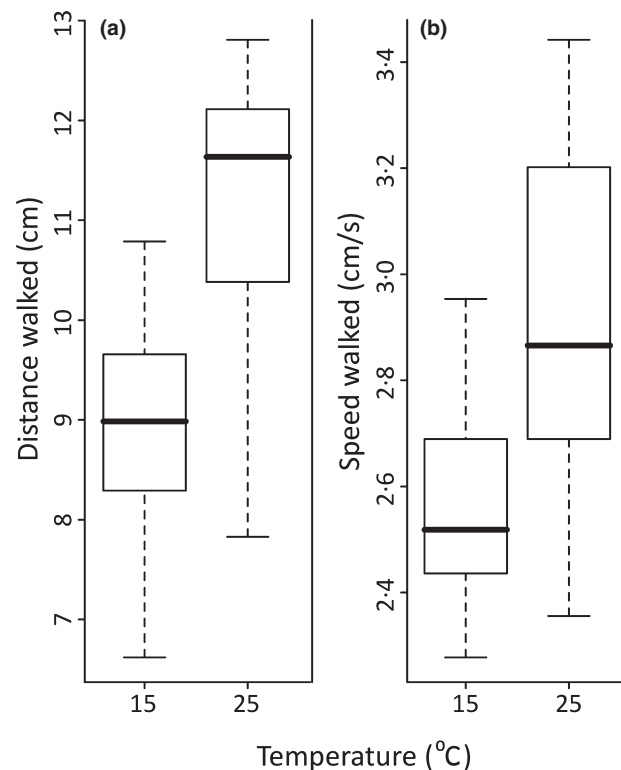


Fig. 3. Comparison of $\ln(x + 1)$ -transformed endurance times: (a) distance walked and (b) speed walked across all individuals at 15 and 25 °C. Solid black lines denote the median value for each cross type and the open vertical rectangles represent the quartiles. The dotted lines extend to the minimum and maximum values. Statistics are presented in Table 3.

Backcrosses and F1 individuals walked significantly farther than CTS (Fig. 4a; Table 3). This general pattern of hybrid vigour was also evident for walking speed, but was not statistically significant for backcross and F1 hybrid walking speed contrasts to CTS (Fig. 4b; Table 3). F2 individuals performed poorly relative to other hybrid individuals, but performed similarly to each parental species with respect to distance walked (Fig. 4a; Table 3). However, we observed a decrease in the walking speed of F2 individuals relative to all other cross types (Fig. 4b; Table 3). We found some support for an interaction of relative mass and line-cross type on the

Table 2. Linear mixed model comparison using corrected Akaike's information criterion (AICc). The best models for each response variable are denoted with an asterisk. All parameters listed in models are included singly in addition to any interactions

Model	d.f.	AICc	
		Distance	Speed
Null	4	316.0815	45.0968
Temperature	5	268.5312*	17.3479*
Line-cross type	9	316.3864	47.0039
Mass	5	317.6934	43.8530
Temperature + Line-cross type	10	259.2560†	15.2200†§
Temperature + Mass	6	270.5434	17.7644
Line-cross type + Mass	10	318.0244	45.4690
Temperature × Line-cross type	15	267.1547	18.7666
Temperature × Mass	7	271.9501	19.3714
Line-cross type × Mass	15	315.7558	48.8171
Temperature + Line-cross type + Mass	11	261.3342	15.4387‡
Temperature × Line-cross type + Mass	16	269.2998	19.1582
Temperature + Line-cross type × Mass	16	257.4974‡§	21.4468
Temperature × Mass + Line-cross type	12	262.9860	16.9398
Temperature × Line-cross type × Mass	27	275.3716	29.0199

*Best single-factor model.

†Best two-factor model.

‡Best three-factor model.

§Best overall model.

distance travelled (Table 3) and there is a significant positive relationship between performance and mass for BTS ($F_{1,3} = 10.2$, $P = 0.05$; Fig. 5). In contrast, CTS and all other line-cross types show no significant relationship between mass and performance after accounting for mass differences between cross types.

For distance travelled we recorded R_{10} values ranging from 4.2 to 27.7 (Table 1) with a mean R_{10} of 12.6. Thus, on average salamanders walked more than 12 times farther at 25 °C than at 15 °C. This difference in distance travelled across temperatures was the greatest for CTS (1082 vs. 39 m at 25 °C and 15 °C respectively, $R_{10} = 27.7$), and the smallest for BTS (682 vs. 161 m, $R_{10} = 4.2$). For speed travelled, however, corresponding Q_{10} values were much lower, ranging from 1.1 for F2 hybrids to 1.7 for F1s (Table 1) and averaging 1.5. Once again, the Q_{10} for CTS (1.6) was on the high end of the observed range and that of BTS on the low end (1.2), suggesting that the two species differ in their response to temperature.

Discussion

EFFECT OF TEMPERATURE ON PERFORMANCE

Temperature appears to be the most important factor influencing locomotor performance (Table 2). The performance differences are very large, supporting the general pattern of increasing locomotor performance with increasing temperature that has been previously observed in ectotherms, including salamanders (Bennett 1990). Else & Bennett (1987) demonstrated a significant positive effect of temperature on running endurance (at 10 °C and 20 °C) for the Arizona tiger salamander (*A. tigrinum nebulosum*), and our data both corroborate their general findings and extend them taxonomically within the tiger salamander complex. However, our R_{10} values are dramatically higher than those found by Else &

Bennett (1987) indicating a more pronounced effect of temperature on endurance in this system, particularly for the native CTS. While some of the differences in R_{10} values can be attributed to methodological differences (for example, we did not systematically increase the speed of the treadmill during trials), our results indicate a much more profound effect of temperature on distance travelled, particularly for animals

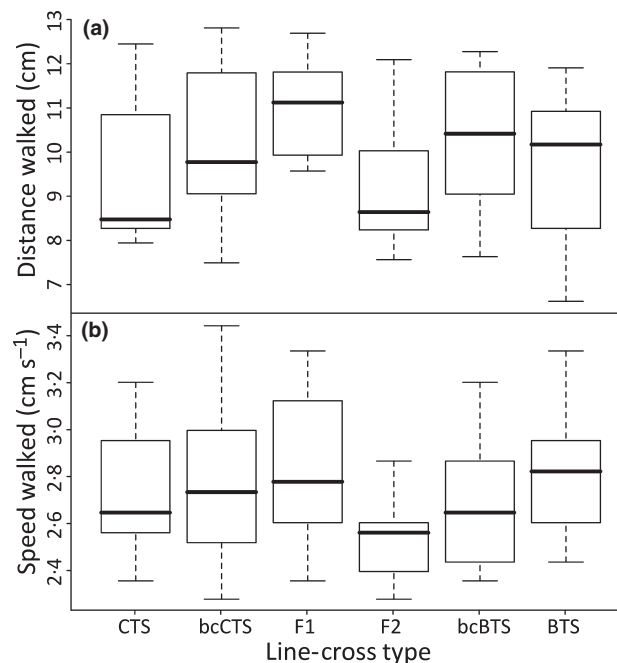


Fig. 4. Box and whisker plot describing the effect of line-cross type on $\ln(x + 1)$ -transformed endurance times across temperatures: (a) distance walked and (b) speed walked. Solid black lines denote the median value for each cross type and the surrounding boxes represent the quartiles. The dotted lines extend to the minimum and maximum values. Statistics are presented in Table 3.

Table 3. Summary of fixed-effects from regression models with the lowest AIC value for each of the three response variables measured

Response	Level	Value	Std.Error	d.f.	<i>t</i> -value	<i>P</i> -value
Distance	Intercept	8.48984	0.31925	68	26.59334	< 0.0001
	Temperature [25]***	2.04058	0.23502	68	8.68246	< 0.0001
	Line-cross type [F1]***	1.52798	0.44027	68	3.47053	0.0009
	Line-cross type [F2]	-0.27364	0.41979	68	-0.65186	0.5167
	Line-cross type [BTS]	0.26706	0.44027	68	0.60657	0.5462
	Line-cross type [bcBTS]**	0.81261	0.38321	68	2.12053	0.0376
	Line-cross type [bcCTS]**	0.75391	0.37547	68	2.00793	0.0486
	Mass	-0.11832	0.11393	68	-1.03844	0.3027
	Line-cross type [F1]:Mass	0.03982	0.16626	68	0.23949	0.8114
	Line-cross type [F2]:Mass	0.02980	0.12325	68	0.24178	0.8097
	Line-cross type [BTS]:Mass*	0.21942	0.11953	68	1.83564	0.0708
	Line-cross type [bcBTS]:Mass	0.12521	0.11839	68	1.05762	0.2940
	Line-cross type [bcCTS]:Mass	0.02833	0.13694	68	0.20689	0.8367
	Speed	Intercept	2.56057	0.07552	74	33.90533
Temperature [25]***		0.33476	0.05397	74	6.20301	< 0.0001
Line-cross type [F1]		0.11275	0.10462	74	1.07773	0.2847
Line-cross type [F2]*		-0.18433	0.09975	74	-1.84786	0.0686
Line-cross type [BTS]		0.09555	0.10462	74	0.91324	0.3641
Line-cross type [bcBTS]		-0.03114	0.09106	74	-0.34194	0.7334
Line-cross type [bcCTS]		0.07849	0.08922	74	0.87977	0.3818

*Significance at $\alpha = 0.1$.

**Significance at $\alpha = 0.05$.

***Significance at $\alpha = 0.01$.

Line-cross type comparisons are made using the CTS value as a reference sample.

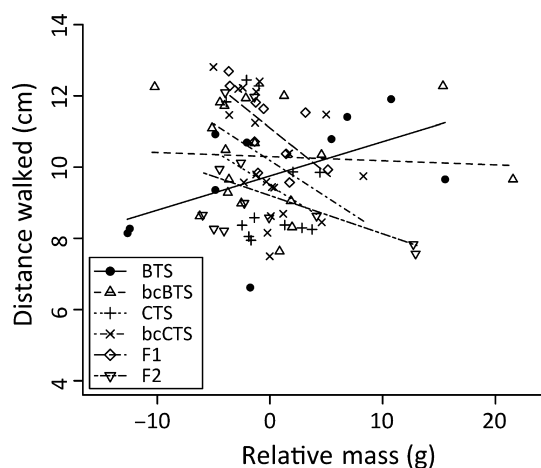


Fig. 5. Scatter plots of the relationship between relative mass and $\ln(x + 1)$ -transformed distance walked. Linear mixed-effect models of the type 'Response ~ Temperature + Mass | ID' for each line-cross type category reveal that only BTS performance is significantly affected by mass ($F_{1,3} = 10.2$, $P = 0.05$). Comparisons of interactions between line-cross types and mass are presented in Table 3.

with a complete (CTS) or nearly complete (bcCTS) native CTS genome. Given the unique life history of CTS, where newly metamorphosed juveniles emerge and migrate at the onset of the hot, dry California summer, this may suggest that physiological adaptation to warm temperatures is an important attribute of CTS success in the Central Valley environment.

Our data demonstrate that CTS, BTS and early-generation hybrids can all walk longer distances, at greater speeds, at higher temperatures. Assuming that walking greater distances

affords new metamorphs an increased opportunity to find a suitable shelter in the few pre-dawn hours available to any given individual, our results imply that delaying metamorphosis as late as possible in the season might constitute an optimal strategy. This would particularly be the case for native CTS, which, although small at metamorphosis, display a nearly 30-fold increase in distance travelled at 25 °C compared to 15 °C. However, selection for metamorphic timing may be also be driven by other factors that select for early emergence, such as rainfall, humidity or competition with conspecifics for terrestrial burrows or food.

The total evidence available from previous studies, which is quite limited, suggests that early metamorphosis at cooler temperatures seems to be the overall preferred strategy for CTS metamorphosis. Based on field (C. Searcy & B. Shaffer, unpublished data), mesocosm (Ryan, Johnson & Fitzpatrick 2009) and laboratory (J.R. Johnson, B.M. Fitzpatrick & H.B. Shaffer unpublished) data, the CTS metamorphosis strategy appears to be: (i) get out as soon as possible or (ii) get out during the first rain after reaching some minimum size threshold. Although from a pure locomotor performance perspective, selection for delayed metamorphosis at a higher temperature seems to be optimal, the short hydroperiods and absence of rainfall typical of CTS breeding sites during spring and summer emergence appears to select for rapid emergence, regardless of cooler temperatures.

EFFECT OF LINE-CROSS TYPE ON PERFORMANCE

Line-cross type was also important in determining the performance of individuals. Temperature and line-cross type did

not significantly interact to affect performance, but we did observe some differences among line-cross types with respect to their response to changes in temperature, as evident from the wide-ranging R_{10} values (Table 1). Hybrid individuals (with the exception of F2s) consistently walked farther than individuals from either parental species. Specifically, F1 animals walked farther than any other cross type (significantly farther, but not faster than CTS), providing yet another example of hybrid vigour in this system. Critically, these are the first published data demonstrating hybrid vigour from the post-metamorphic salamander life history stage for CTS-BTS hybrids. Similar studies of fishes (Rosenfield *et al.* 2004) and lizards (Kearney, Wahl & Autumn 2005) have also found hybrid vigour with respect to locomotor performance. Predictably, F2 individuals in our study appear to suffer from hybrid dysfunction following the disruption of co-adapted gene complexes during the first generation of recombination (e.g. Lynch 1991).

Our data suggest that large differences in performance based on the genetic composition of an individual's genome have the potential to affect the fitness of particular genotypic classes (i.e. F1, bcBTS, bcCTS) and enhance the spread of the hybrid swarm. For example, landscape genetics of CTS has identified genetic structure resulting from isolation-by-distance and terrestrial habitat composition (Wang, Savage & Shaffer 2009). If hybrid individuals are capable of travelling farther than native CTS, they have the potential to disrupt natural patterns of population structure and spread non-native alleles deeper into the range of CTS. Ecological theory and empirical data also suggest that long-distance dispersers play a dominant role in determining the rate of spread of invasive species (Neubert & Caswell 2000). F1 hybrids in this system appear to be primed for long-distance dispersal. Very recent genetic data may further support this view; the populations studied by Wang, Savage & Shaffer (2009) have a low level of non-native genes present, suggesting a more efficient movement of non-native genes on the landscape than was previously thought (Fitzpatrick *et al.* 2010).

EFFECT OF MASS ON PERFORMANCE

Bennett, Garland & Else (1989) found greater performance capacity of larger *A. tigrinum* salamanders and we expected to find similar results. However, we found no overall effect of mass on performance. We did identify a positive relationship between mass and performance for the introduced *A. t. mavortium*, which are genetically similar to the *A. t. nebulosum* salamanders used by Bennett, Garland & Else (1989). For all other genotypic categories, we did not detect a significant effect and in most cases, the relationship appeared negative (Fig. 5). The lack of significant relationships observed for non-BTS line-cross types may reflect real biological differences between *A. californiense* and other members of the tiger salamander complex or it may reflect the much smaller range of body mass examined in our study compared with that in Bennett, Garland & Else (1989).

Conclusion

Overall, our results suggest an advantage to late metamorphosis in terms of temperature-dependent locomotor ability, although other trade-offs, such as increased desiccation risk related to metamorphic timing, remain uninvestigated. We did not predict this advantage given the reliably short times to metamorphosis of native CTS, and it provides the greatest benefit to hybrid and introduced individuals that routinely delay metamorphosis, particularly in artificially enhanced ponds with long hydroperiods. Native, introduced and hybrid tiger salamanders appear to have the capacity to move much farther at higher temperatures, with potential benefits in terms of finding suitable terrestrial refugia.

While we did not find significant interactions between line-cross type and temperature, the performance of F1 hybrids (and backcrosses to a lesser degree) suggests an enhanced capacity for spread of introduced genes relative to the typical dispersal distances of native CTS across a range of temperatures. The primary question for future research are whether an increased capacity for movement results in longer movements of hybrids in natural populations, and how these movements affect population structure and the spread of non-native alleles. Low-levels of non-native genes have already been detected across a large part of the CTS range (Fitzpatrick *et al.* 2009, 2010), and levels of introgression are particularly high in human-modified, relatively permanent aquatic habitats that allow for late-emerging hybrid metamorphs (Fitzpatrick & Shaffer 2007b). This improved understanding of the effect of abiotic environmental variation on the movements of salamanders will aid in predicting the consequences of global climate change on connectivity and population dynamics, a critical element in managing this biological invasion.

Acknowledgements

We thank L. Austin for contributing the neoprene for the treadmill and the Shaffer Lab group for helpful discussion. Additionally, we thank B. Fitzpatrick, J. Piovia-Scott, M. Ryan and three anonymous reviewers for comments on early manuscript drafts. All procedures were approved by the UC Davis Animal Care and Use Committee (protocol #13269). This work was supported by a REU supplement from the National Science Foundation (DEB#0516475) and the UC Davis Agricultural Experiment Station.

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Received 25 January 2010; accepted 6 April 2010

Handling Editor: Raoul Van Damme

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1. Average daily temperatures.

Fig. S2. Treadmill design specifications.

Fig. S3. Line-cross type mass boxplots.

Fig. S4. Temperature Interaction plots.

Table S1 Log-likelihood model comparisons.

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