Trade-offs between baseline thermal tolerance and thermal tolerance plasticity are much less common than it appears

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Running header: Constraints on plasticity

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Abstract

Thermal tolerance plasticity is a core mechanism by which organisms can mitigate the effects of climate change. As a result, there is a need to understand how variation in tolerance plasticity arises. The Baseline Tolerance/Plasticity Trade-off Hypothesis (hereafter referred to as the Trade-off Hypothesis, TOH) has recently emerged as a potentially powerful explanation. The TOH posits that organisms with high baseline thermal tolerance have reduced thermal tolerance plasticity relative to those with low baseline tolerance. Many studies have found support for the TOH. However, this support must be regarded cautiously because the most common means of testing the TOH can yield spurious 'trade-offs' due to regression to the mean. I acquired data for 25 previously-published analyses that supported the TOH at the intraspecific level and reanalyzed them after applying a method that adjusts plasticity estimates for regression to the mean. Only four of the 25 analyses remained statistically significant after adjustment, and effect size and variance explained decreased in all cases. The few data sets in which support for the TOH was maintained after adjustment point to areas of future study, but are too few to make generalizations at this point. In sum, regression to the mean has led to a substantial overestimation of support for the TOH and must be accounted for in future tests of the hypothesis.

Introduction

The capacity to physiologically adjust to temperature change through phenotypic plasticity is thought to influence vulnerability to anthropogenic warming (Gunderson, Dillon, & Stillman, 2017; Huey et al., 2012; Morley, Peck, Sunday, Heiser, & Bates, 2019; Seebacher, White, & Franklin, 2015; Somero, 2010). Uncovering how and why taxa differ in physiological plasticity is therefore of great interest (Kelly, 2019; Pottier, Burke, Drobniak, Lagisz, & Nakagawa, 2021; Rohr et al., 2018). Recently, the Trade-off Hypothesis (TOH) has emerged as a potentially powerful mechanism to explain variation in the plasticity of thermal tolerance limits (Stillman, 2003; van Heerwaarden & Kellermann, 2020). This hypothesis posits that taxa with high baseline levels of thermal tolerance will exhibit low levels of thermal tolerance plasticity. From a mechanistic perspective, the TOH is usually discussed in terms of phenotypic constraint. Thermal limits can only be so high, and therefore plasticity must be reduced as baseline tolerance rises. The implications of this hypothesis, if and when true, are many, and touch not just on global change biology but fundamental aspects of physiological ecology and evolution (reviewed in van Heerwaarden & Kellermann, 2020).

Support for the TOH has been found in several studies at both the intraspecific and interspecific levels (Barley et al., 2021; Comte & Olden, 2017; van Heerwaarden & Kellermann, 2020; see also Table 1). Nonetheless, these results must be regarded carefully. This is because many tests of the TOH open the door for regression to the mean to generate spurious support (van Heerwaarden & Kellermann, 2020). Baseline thermal tolerance values are used to calculate plasticity, and then plasticity is modeled as a function of the baseline value. A common term is

present on both sides of the function, potentially leading to a negative relationship that is a statistical artifact.

Approaches for identifying, avoiding, and/or correcting for regression to the mean exist, but to date have rarely been applied to tests of the TOH. O'Neill, Davis, & MacMillan (2021) tentatively found support for the TOH among *Drosophila melanogaster* individuals with respect to cold tolerance, but from the correlation structure within their data, they inferred that the relationship was probably artifactual. Deery et al. (2021) found initial support for the TOH at the individual level among *Anolis* lizards with respect to heat hardening, but subsequently conducted a data resampling analysis (Ghalambor et al., 2015; Jackson & Somers, 1991) and concluded that the relationships were spurious. Finally, Gunderson & Revell (2022) used simulations to show how pervasive regression to the mean is when testing the TOH at macroevolutionary scales. They implemented a post-hoc method for adjusting plasticity data to account for regression to the mean (Kelly & Price, 2005), and found that doing so reduced support for the TOH in a previously-published dataset (Armstrong, Tanner, & Stillman, 2019; Gunderson & Revell, 2022). In sum, studies that attempt to account for regression to the mean in tests of the TOH have generally found that support for the hypothesis declines. How general is this outcome?

Methods

I reanalyzed data from published studies that tested and found support for the TOH, focusing on work conducted at the intraspecific level (see Gunderson & Revell (2022) for analysis of the same issues at a macroevolutionary scale). I started with data from the studies summarized in

88 Table 1 of van Heerwaarden & Kellermann (2020), and added as many subsequently-published

89 data sets as I could identify. Data for some studies could not be obtained or the analysis

90 supporting the TOH did not allow for correction. In total, I reanalyzed 25 correlations between

91 baseline thermal tolerance and thermal tolerance plasticity representing 10 species (Table 1). I

92 first conducted correlation analyses on the original data ("unadjusted"), and then with plasticity

93 values adjusted to remove the effect of regression to the mean following the method

94 developed by Kelly & Price (2005; "adjusted"). Detailed explanation of the method can be found

Table 1. Summary of the data sets reanalyzed in the present study. "Unadjusted" correlation coefficients were calculated using raw plasticity data as in the original analyses, and "Adjusted" correlation coefficients were calculated after removing the effect of regression to the mean (Kelly and Price 2005). Correlation coefficients (*r*) in bold indicate statistically significant relationships at the α = 0.05 level. If a study conducted multiple analyses with different subsets of their data, the subset is indicated by the "Sample" column. The "Metric" definitions are as follows: *CTmax* = critical thermal maximum; *LD50* = temperature of 50% mortality; *CCRT* = chill coma recovery time; *CCO* = chill coma onset.

Clade	Species	Level	Metric	Sample	Unadjusted *r*	Adjusted *r*	Reference
Heat tolerance							
Fish	*Gasterosteus aculeatus*	Individual	CTmax	-	**-0.59**	0.02	Mottola et al. 2022
Fish	*Danio rerio*	Individual	CTmax	Females	**-0.70**	-0.09	Morgan et al. 2018
Fish	*D. rerio*	Individual	CTmax	Males	**-0.80**	0.02	Morgan et al. 2018
Reptile	*Anolis sagrei*	Individual	CTmax	-	**-0.55**	0.04	Deery et al. 2021
Reptile	*A. carolinensis*	Individual	CTmax	-	**-0.65**	0.00	Deery et al. 2021
Reptile	*Lampropholis coggeri*	Individual	CTmax	Observer 1	**-0.77**	**-0.49**	Phillips et al. 2016
Reptile	*L. coggeri*	Individual	CTmax	Observer 2	**-0.52**	-0.11	Phillips et al. 2016
Reptile	*Urosaurus ornatus*	Individual	CTmax		**-0.48**	-0.07	Glbert and Miles 2019
Insect	*Drosophila melanogaster*	Population	CTmax	-	**-0.73**	**-0.52**	van Heerwaarden et al. 2016
Crustacean	*Acartia tonsa*	Population	LD50	-	**-0.84**	-0.06	Sasaki and Dam 2019
Crustacean	*A. tonsa*	Population	LD50	-	**-0.97**	0.66	Sasaki and Dam 2020
Crustacean	*A. tonsa*	Selection line	LD50	CT pop., control	**-0.99**	0.54	Sasaki and Dam 2021
Crustacean	*A. tonsa*	Selection line	LD50	CT pop., warming	**-0.94**	-0.38	Sasaki and Dam 2021
Crustacean	*A. tonsa*	Selection line	LD50	SP pop., control	**-1.00**	0.84	Sasaki and Dam 2021
Crustacean	*A. tonsa*	Selection line	LD50	SP pop., warming	**-0.99**	**-0.99**	Sasaki and Dam 2021
Crustacean	*A. tonsa*	Selection line	LD50	PG pop., control	**-0.97**	0.13	Sasaki and Dam 2021
Crustacean	*A. tonsa*	Selection line	LD50	PG pop., warming	**-0.98**	**-0.97**	Sasaki and Dam 2021
Cold tolerance							
Insect	*D. melanogaster*	Female lines	CCRT	Low heat	**-0.81**	0.01	Noh et al. 2017
Insect	*D. melanogaster*	Female lines	CCRT	Mid heat	**-0.77**	-0.04	Noh et al. 2017
Insect	*D. melanogaster*	Female lines	CCRT	High heat	**-0.87**	0.02	Noh et al. 2017
Insect	*D. melanogaster*	Individuals	CCRT	15°C Acclimated	**-0.68**	-0.09	O'Neill et al. 2021
Insect	*D. melanogaster*	Individuals	CCRT	25°C Acclimated	**-0.62**	-0.03	O'Neill et al. 2021
Insect	*D. melanogaster*	Individuals	CCO	15°C Acclimated	**-0.63**	0.00	O'Neill et al. 2021
Insect	*D. melanogaster*	Individuals	CCO	25°C Acclimated	**-0.62**	0.01	O'Neill et al. 2021
Insect	*Aphaenogaster picea*	Populations	CCRT	-	**-0.88**	0.04	Nguyen et al. 2019

in Kelly & Price (2005) and Gunderson & Revell (2022). All analyses were conducted in R (R Development Core Team, 2021). Data adjustment was done with a function I wrote in R ("rttm.adj"), and the script can be found in the Supplementary Materials.

Results and Discussion

Two examples of what data look like before and after adjustment for regression to the mean are shown in Figure 1. The first example (Figure 1 A, B) shows heat hardening data for

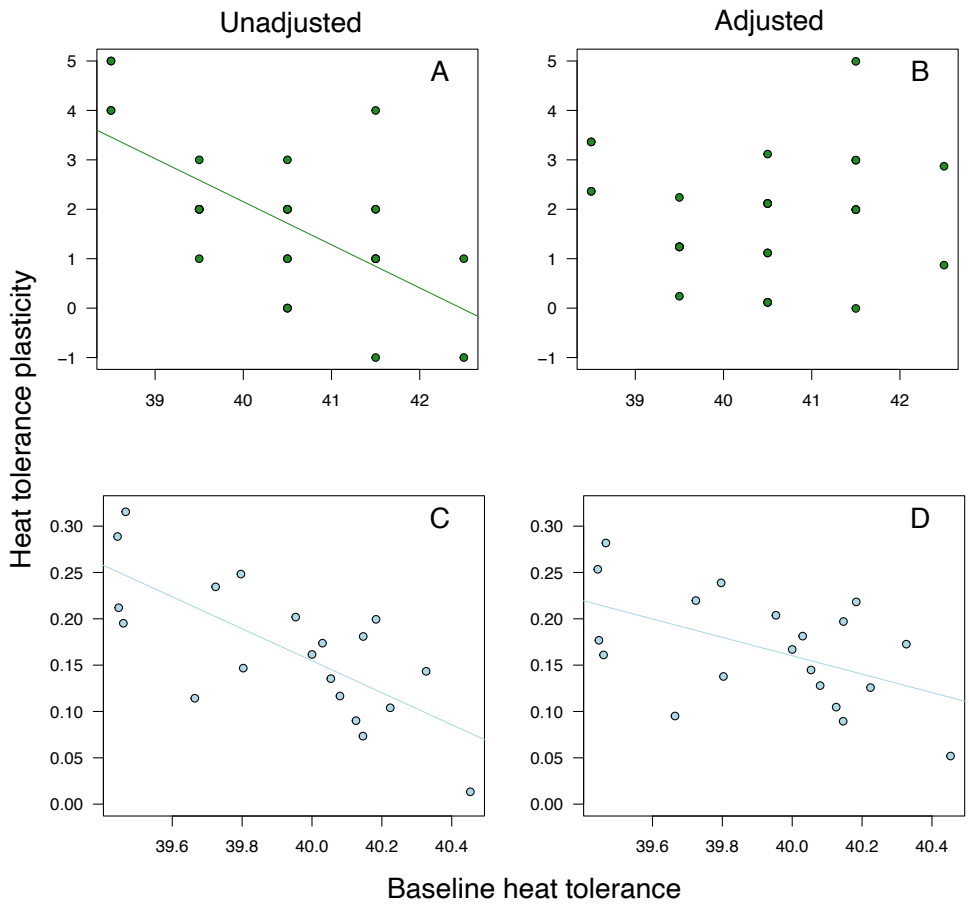

Figure 1. Examples of how tests of the Trade-off Hypothesis (TOH) are affected when plasticity values are adjusted to account for regression to the mean using the Kelly & Price (2005) method. A, B). Heat hardening data from the lizard *Anolis carolinensis* (Deery, Rej, Haro, & Gunderson, 2021). Support for the TOH was lost after data adjustment. C,D) Heat tolerance acclimation data from adult *Drosophila melanogaster* (van Heerwaarden, Kellermann, & Sgrò, 2016). Support for the TOH was maintained after data adjustment, though the effect size and variance explained decreased.

individuals of the lizard *Anolis carolinensis* (Deery et al., 2021). In the unadjusted data (Figure 1 A), there is a strong negative relationship between an individual's baseline heat tolerance limit and how much their heat tolerance limit increases after heat shock (i.e., plasticity). However, that relationship disappears after plasticity values are adjusted for regression to the mean (Figure 1 B). The second example shows data on heat tolerance acclimation in *Drosophila melanogaster* (van Heerwaarden et al., 2016). In this case, a negative relationship between baseline tolerance and plasticity is maintained in both the unadjusted and adjusted data (Figure 1 C, D), though the effect of regression to the mean is still noticeable: the correlation coefficient (and therefore variance explained) and the effect size (slope) are all smaller using the adjusted versus the unadjusted plasticity data.

When this approach is applied across all the data sets, it becomes apparent that most relationships between baseline thermal tolerance and thermal tolerance plasticity are spurious due to regression to the mean (Table 1). Of the 25 results consistent with the TOH using unadjusted data, only four remain statistically significant at the $\alpha = 0.05$ level after adjustment of plasticity values. Furthermore, correlation coefficients decrease in almost every case whether statistical significance is maintained or not. The magnitude and sometimes direction of effect also changes considerably after data adjustment. Slopes of the relationship between baseline tolerance and tolerance plasticity for all data sets become less negative when using adjusted versus unadjusted plasticity values (Figure 2).

Some of the regression to the mean effects demonstrated here are consistent with conclusions drawn by the original authors using different approaches (e.g., Deery et al., 2021; O'Neill et al., 2021; see above). However, most of the initial, unadjusted analyses were

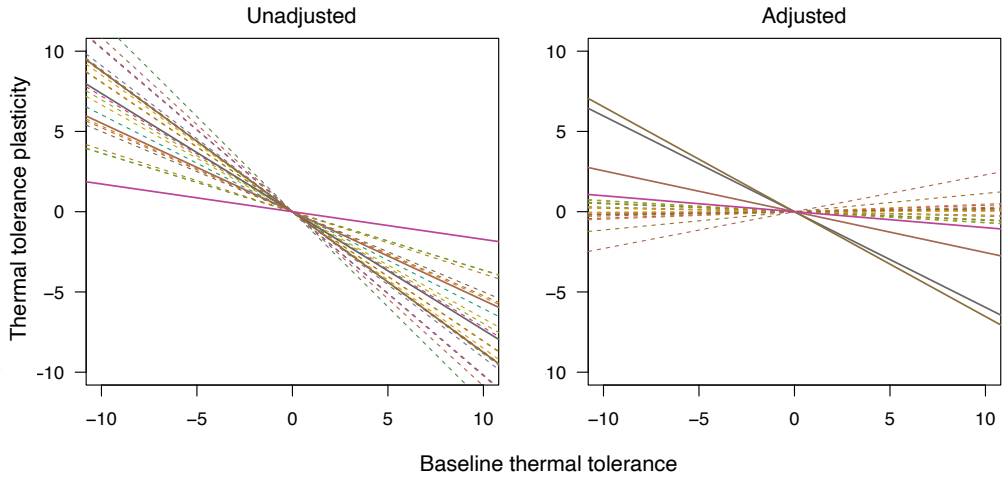

Figure 2. Slopes for the relationship between baseline thermal tolerance and thermal tolerance plasticity for the data sets in Table 1. Intercepts were set a 0 so all relationships could easily be visualized together. *Left*: Slopes when thermal tolerance plasticity values are not adjusted for regression to the mean. *Right*: Slopes when thermal tolerance plasticity values are adjusted for regression to the mean. Solid lines: data sets for which significance was maintained at $\alpha = 0.05$ after data adjustment. Dashed lines: data sets for which statistical significance was not maintained after data adjustment.

interpreted as evidence consistent with the TOH. Comparing data sets for which support for the TOH was and was not maintained after data adjustment is informative. Support for the TOH was rarely maintained after data adjustment in individual-level studies (Table 1). The lone example was with respect to heat hardening in the lizard *Lampropholis coggeri* (Phillips et al., 2016). Interpretation of this result is not straightforward, however, because it is not consistent with other data from the same species in the same study: two different observers measured thermal tolerance values, and support for the TOH was maintained after adjustment with data from one observer, but not the other (Table 1).

The other data sets for which support for the TOH was maintained came from a study of trade-offs between developmental and adult plasticity in *Drosophila melanogaster* (van Heerwaarden et al., 2016) and an experimental evolution study with the copepod *Acartia tonsa* (Sasaki & Dam, 2021). In the *Drosophila* study, animals were exposed to different

136 developmental temperatures, after which these same animals had their capacity for adult
137 thermal acclimation measured. The authors found that development in warm conditions led to
138 increased baseline thermal tolerance, but lower capacity for adult acclimation (van
139 Heerwaarden et al., 2016). These data are consistent with a mechanistic link between
140 developmental and adult plasticity (Beaman, White, & Seebacher, 2016; van Heerwaarden et
141 al., 2016).

142 In the experimental evolution study with the copepod *A. tonsa*, control and high
143 temperature lines were established from three different source populations (Sasaki & Dam,
144 2021). In the initial analysis, there was an apparent trade-off between baseline thermal
145 tolerance and plasticity over time across all control and high temperature groups from all three
146 source populations. However, data adjustment shows that the relationship was artifactual in all
147 three of the control groups, but not in two of the three high temperature selection groups
148 (Table 1). In the third high temperature selection group, the correlation coefficient remained
149 relatively large and negative after data adjustment (-0.38), though statistical significance was
150 not maintained (Table 1). In sum, the data are consistent with a real evolutionary correlation
151 between baseline tolerance and plasticity as populations adapt to warmer conditions (Sasaki &
152 Dam, 2021). However, the generality of this conclusion is not clear even within this species, as
153 the TOH is not supported after data adjustment in a different study focused on natural
154 populations evolving under different thermal regimes (Sasaki & Dam, 2019; Table 1).

155

156 **Conclusion**

Due to regression to the mean, support for the Trade-off Hypothesis is much lower than it appears at first glance (Table 1; see also Sgro et al. (2010) and van Heerwaarden, Lee, Overgaard, & Sgro (2014), who report analyses that reject the TOH with unadjusted data). Studies in which support for the TOH is maintained after data adjustment point to situations in which trade-offs between baseline tolerance and plasticity may be most common. However, at this point there are too few examples to make generalizations. More tests of the TOH are certainly warranted. At the core, the inflated support for the TOH is a problem of identifying the appropriate null expectation (Hertz, Huey, & Stevenson, 1993). A lack of association between baseline thermal tolerance and thermal tolerance plasticity is not always the correct null hypothesis based on the methodological approaches taken (Gunderson & Revell, 2022). By recognizing such situations and accounting for them, we can more quickly develop a clear picture of the forces that influence the evolution of thermal tolerance plasticity, and in what situations different mechanisms are important. Doing so will greatly increase our understanding of organismal responses to ongoing anthropogenic warming.

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Data availability

The data for this paper will be made available in Dryad.

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