© 2015. Published by The Company of Biologists Ltd | The Journal of Experimental Biology (2015) 218, 500 doi:10.1242/jeb.119933

CORRECTION

Temporal patterning of thermal acclimation: from behavior to membrane biophysics

George Somero

There was an error published in a recent Classics article in *J. Exp. Biol.* **218**, 167-169.

George Somero inadvertently referred to one of the authors of the paper under discussion with the incorrect first name. The author's correct name is Michael Friedlander.

We apologise to Michael Friedlander for this error and any inconvenience it may have caused.

© 2015. Published by The Company of Biologists Ltd | The Journal of Experimental Biology (2015) 218, 167-169 doi:10.1242/jeb.109843

CLASSICS

Temporal patterning of thermal acclimation: from behavior to membrane biophysics

George Somero discusses Andy Cossins, James Friedlander and C. Ladd Prosser's classic paper, 'Correlations between behavioral temperature adaptations of goldfish and the viscosity and fatty acid composition of their synaptic membranes', published in *The Journal of Comparative Physiology* in 1977.

In the opening paragraphs of papers focused on biological effects of climate change, one commonly finds a statement to the effect that organisms face only four options in coping with our planet's rapidly rising temperatures: migrate, acclimatize, evolve or perish. The set of options open to a particular species depends on several factors, including generation time, the amount of genetic variation present to allow evolutionary change, and – perhaps most importantly for the short run – a capacity to alter the phenotype through acclimatization (Somero, 2010). For metazoans that have long generation times and limited opportunities to escape rising temperatures through migration, acclimatization may be key to survival. It is thus important to more fully characterize acclimatory processes in ectothermic species, in order to better understand these processes in terms of their kinetics, limits and importance at multiple levels of biological organization – from molecules to behavior. Unfortunately, the existing acclimation literature often manifests significant shortcomings. Too often, studies of laboratory acclimation involve only a single sampling time, which precludes determination of the kinetics of the processes of interest. A further shortcoming of most acclimation studies is their narrow focus in terms of levels of biological organization that are examined. Physiological or biochemical changes generally are not examined in the context of effects at higher levels of organization, such as behavior, which may be the critical indices for evaluating the importance of underlying biochemical and physiological changes on the organism's success and survival. The 1977 paper in *The Journal of Comparative Physiology* on thermal acclimation in goldfish by Andy Cossins, James Friedlander and C. Ladd Prosser (Cossins et al., 1977) stands as a landmark paper in the analysis of acclimation because it presents an integrated perspective that combines information on the time courses of acclimation to both high (25°C) and low (5°C) temperatures in three behavioral responses, synaptic membrane biophysics and lipid membrane lipid chemistry (Fig. 1). The paper elucidates key elements in thermal acclimation and presents a paradigm of how acclimation studies should be done. It is a paper that needs to be brought to the attention of biologists who address issues in climate change today.

This study was a logical outgrowth of the broad experimental program on thermal acclimation in fish that existed in Ladd Prosser's laboratory at the University of Illinois. Work by Bruce Sidell (a PhD student in the laboratory) and colleagues provided some of the first information on acclimatory time course in enzymes of energy metabolism (Sidell et al., 1973). Jeffrey Hazel and Prosser had also started to map out the type of changes in cellular lipids that accompanied thermal acclimation in ectotherms (Hazel and Prosser, 1974). A background for the study of temperature acclimatory changes in fish membrane lipids, notably alterations in double-bond content (saturation) and length of acyl chains, was provided by studies from another laboratory that focused on thermal acclimation by bacteria (Sinensky, 1974). These microbial studies led to the concept of 'homeoviscous' adaptation – the conservation of a consistent viscosity (static order) of membrane lipids following thermal acclimation. Cossins extended this type of analysis to goldfish, a eurythermal fish that has been an important 'lab rat' in studies of acclimation (Cossins, 1977). Friedlander and colleagues, working in the Prosser laboratory, had shown that thermal acclimation affected cerebellar function in goldfish, a neurological change with marked behavioral consequences (Friedlander et al., 1976). Against this backdrop, an integrated study of thermal acclimation to high and low temperatures that comprised a time-course analysis of changes in behavior, synaptic membrane biophysics and membrane lipid chemistry was begging to be done.

In their 1977 paper, Cossins and his colleagues wove these complementary lines of research into an integrated whole (Cossins et al., 1977). They discovered strong correlations among the changes in all three variables and provided a compelling case for the importance of homeoviscous adaptation in synaptic membranes [isolated brain synaptic vesicles (synaptosomes)] in achieving thermal acclimation of neural function and behavior (Fig. 1). All three behavioral traits examined – coma, equilibrium loss and hyper-excitability – showed the same time courses of acclimation, which generally paralleled changes in synaptic membrane static order, as measured using fluorescence polarization of the probe 1,6-diphenyl hexatriene (DPH). A high polarization signal indicates a high static order. Their results provide insights into the relative rates of acclimation at high and low temperatures. As predicted, on the basis of the temperature dependence of physiological processes (Q_{10} effects), acclimation to the higher temperature occurred much more quickly than acclimation to cold. Thus, in the case of the three behavioral traits, a new steady state was reached within about 4 days when fish were transferred from 5°C to 25°C, but after transfer from 25°C to 5°C, a new steady state required almost 28 days. The authors emphasize that the small standard errors of the mean found in their fluorescence polarization measurements show how highly

Classics is an occasional column, featuring historic publications from the literature. Written by modern experts in the field, these articles discuss each classic paper's impact on the field of biology and their own work.

The Journal of Experimental Biology (2015) doi:10.1242/jeb.109843

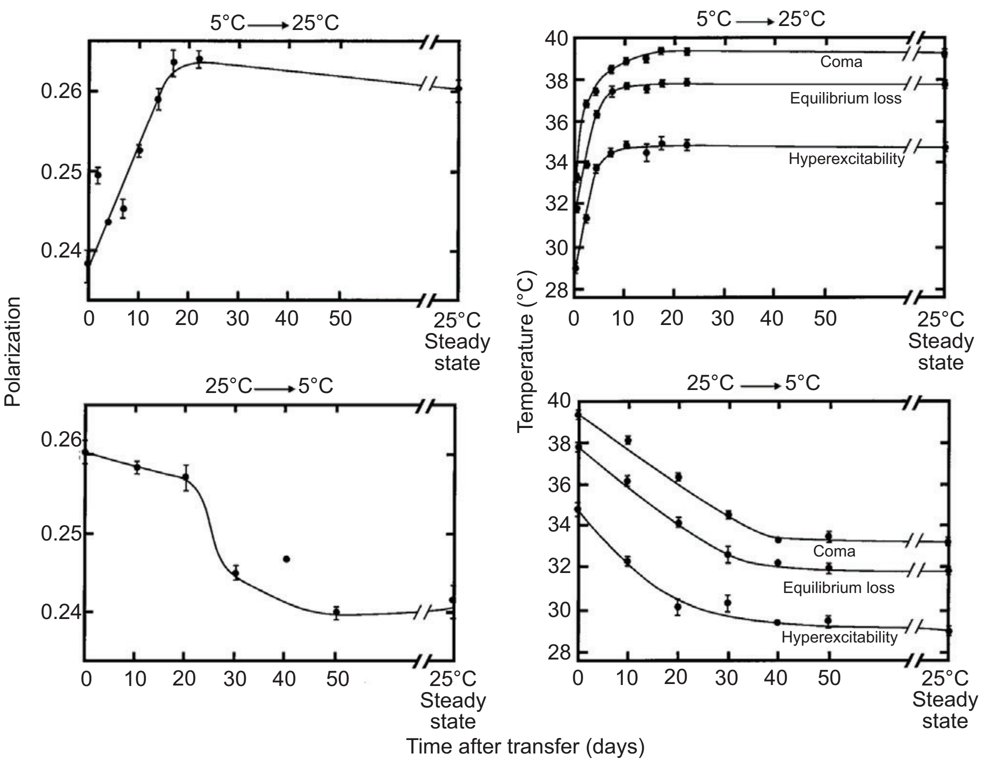

Fig. 1. Time courses of cold and warm acclimation of three behavioral traits and synaptosomal membrane fluidity in the goldfish *Carassius auratus*. Warm (transfer from 5°C to 25°C; top two frames) and cold (transfer from 25°C to 5°C; bottom two frames) acclimation of three behavioral traits (right panels) and synaptosomal membrane fluidity (indexed by fluorescence polarization) (left panels). The fluorescence polarization signal, which is inversely related to membrane fluidity, was measured at a common temperature of 25°C. Figure modified after Cossins et al. (Cossins et al., 1977).

conserved the static order of synaptosomal membranes must be, if synaptic transmission is to be normal. Such low variation is not merely a sign of good technique; it also indicates that close conservation of particular values for a trait is important for sustaining function at different temperatures. Similar arguments have been made in terms of conservation of functional and structural properties of proteins where, again, conservation of a particular balance between rigidity and flexibility of structure at different body temperatures is key to activity (Hochachka and Somero, 2002; Fields et al., 2015). The reported changes in lipid composition in isolated brain synaptosomes, notably, the temperature-dependent changes in acyl chain saturation in phospholipids with different head groups, round out this study by providing a detailed chemical mechanism of the homeoviscous adaptation process.

What effects did this landmark study have on subsequent studies of thermal acclimation and evolutionary adaptation of membrane function? An important extension of this line of work was published in this journal by Logue and colleagues (Logue et al., 2000). The paper, on which Cossins was a co-author, shows that the types of differences in synaptosomal fluidity and composition found in the intra-specific study of goldfish reflects a phylogenetically and environmentally broad pattern. Logue and colleagues examined vertebrates whose normal body temperatures spanned a range of slightly more than 40°C (from Antarctic notothenioid fish living near –1.9°C to birds with core temperatures near 39°C) and discovered a pattern that mirrored the one found in the 1977 acclimation study, namely a close conservation of synaptosomal fluidity at normal body temperatures mediated by alterations in lipid composition. Phenotypic acclimation and evolutionary adaptation thus lead to the same type of temperature-compensatory strategies in these membrane systems. More recent comparative work has demonstrated the general significance of the homeoviscous adaptation response across a wide range of taxa; we now appreciate the significance of this type of adaptation in all domains of life and have a deeper understanding of the types of changes in membrane lipids that underlie the homeoviscous response. Newer work has been successful in taking the analysis down to the level of gene expression. For instance, later work in Cossins' laboratory on the time course of transcriptional changes during acclimation to cold has documented temporal variation in the expression of genes encoding proteins such as desaturases, which are essential for adjusting lipid acyl chain saturation (Gracey et al., 2004). Despite these sorts of progress, however, I am aware of no subsequent study that provides the integrative analysis of the 1977 paper, which cuts across levels of biological organization and presents a temporal analysis that is commonly lacking in acclimation studies.

What might be profitable areas for future studies of acclimation of membrane systems? One issue concerns the question of whether the membrane proteome in differently acclimated specimens changes during acclimation. Is the acclimatory effort shared between proteins and lipids, or do only the latter constituents bear the burden of adaptation? Information on the possible role of coupled acclimatory changes in the lipid composition and the proteome of membranes would be valuable to give a more inclusive picture of how membrane function is sustained at different temperatures. In the context of climate change, it will be important to examine diverse ectothermic species that have evolved in environments with different amounts of thermal variation, to learn more about the ranges of temperature over which homeoviscous adaptation can be achieved. Do stenothermal species that have evolved in thermally stable environments have a truncated range of temperatures over which this process can occur, compared with eurythermal species? How close do current maximal body temperatures of a species lie to the temperatures at which further homeoviscous adaptation is impossible?

In summary, the 1977 study by Cossins, Friedlander and Prosser has a great deal to offer in terms of its contribution to our understanding of thermal acclimation and for teaching us how best to conduct studies of acclimation. I feel it is especially critical to emphasize the importance of time-course studies of acclimation. This caveat may apply especially to studies of environmental stress that utilize transcriptomic and proteomic methodologies. Here, perhaps because of the costs of these experiments, taking multiple samples at several time points is not commonly done. A ‘snapshot’ of a stressor-induced response in the transcriptome or proteome taken at a single time point cannot provide an adequate picture of the process of interest. Furthermore, circadian rhythms in gene expression intertwine with stress-induced effects on transcription, further limiting insights from ‘snapshot’ analyses (Connor and Gracey, 2011). The 1977 paper by Cossins and colleagues thus has an important and still current methodological caveat for those who examine the responses of organisms to stress.

I hope that my enthusiasm for this paper is infectious and leads the reader to examine this classic, which is coming close to its 40th anniversary. This publication is well worth tracking down and it should serve as a basis for thinking about how experimentation on environmental change should be done.

George Somero
Stanford University
somero@stanford.edu

References

Connor, K. M. and Gracey, A. Y. (2011). Circadian cycles are the dominant transcriptional rhythm in the intertidal mussel Mytilus californianus. *Proc. Natl. Acad. Sci. USA* **108**, 16110-16115.

Cossins, A. R. (1977). Adaptation of biological membranes to temperature: Effect of seasonal acclimation of goldfish upon the viscosity of synaptosomal membranes. *Biochim. Biophys. Acta* **470**, 395-411.

Cossins, A. R., Friedlander, M. J. and Prosser, C. L. (1977). Correlations between behavioural temperature adaptations of goldfish and the viscosity and fatty acid composition of their synaptic membranes. *J. Comp. Physiol.* **120**, 109-121.

Fields, P. A., Dong, Y. , Meng, X. and Somero, G. N. (2015). Adaptation of protein structure and function to temperature: there is more than one way to skin a cat. *J. Exp. Biol.* (in press).

Friedlander, M. J., Kotchabhakdi, N. and Prosser, C. L. (1976). The effects of heat and cold on behavior and cerebellar function in goldfish. *J. Comp. Physiol.* **112**, 19-45.

Gracey, A. Y., Fraser, E. J. , Li, W., Fang, Y., Taylor, R. R., Rogers, J. , Brass, A. and Cossins, A. R. (2004). Coping with cold: An integrative, multi-tissue analysis of the transcriptome of a poikilothermic vertebrate. *Proc. Natl. Acad. Sci. USA* **101**, 16970-16975.

Hazel, J. R. and Prosser, C. L. (1974). Molecular mechanisms of temperature compensation in poikilotherms. *Physiol. Rev.* **54**, 620-677.

Hochachka, P. W., and Somero, G. N. (2002). *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. New York: Oxford University Press.

Logue, J. A., DeVries, A. L., Fodor, E., and Cossins, A. R. (2000). Lipid compositional correlates of temperature-adaptive interspecific differences in membrane physical structure. *J. Exp. Biol.* **203**, 2105-2115.

Sidell, B. D., Wilson, F. R., Hazel, J., and Prosser, C. L. (1973). Time course of thermal acclimation in goldfish. *J. Comp. Physiol.* **84**, 119-127.

Sinensky, M. (1974). Homeoviscous adaptation – A homeostatic process that regulates the viscosity of membrane lipids in Escherichia coli. *Proc. Natl. Acad. Sci. USA* **71**, 522-535.

Somero, G. N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine ‘winners’ and ‘losers’. *J. Exp. Biol.* **213**, 912-920.