Journal of Zoology

ZSL LET'S WORK FOR WILDLIFE

Journal of Zoology. Print ISSN 0952-8369

RESPONSE

Response to the Werner (2016) comment on 'Polyploid unisexual salamanders have higher tissue regeneration rates than diploid sexual relatives'

R. D. Denton

Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus, OH, USA

doi:10.1111/jzo.12397

Correspondence
Robert D. Denton, Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus, OH, USA.
Tel: 614 292 2891
Email: robert.d.denton@gmail.com

In his comment on Saccucci *et al.* (2016), Yehudah Werner (2016) suggests that differences in regeneration rate between diploid and polyploid salamanders could simply be a result of differences in thermal optima for each group relative to the temperatures at which the experiments were conducted. While this is possible, I feel that this explanation is unlikely to account for the large difference observed in regeneration rates for the following three reasons.

Similar thermal preferences among study species and related groups

Thermal preferences for *Ambystoma texanum* and the species that is most represented in the unisexuals in this study, *A. jeffersonianum*, differ by slightly more than one degree (26.5°C for *A. texanum*; Dupré & Petranka, 1985 and 25.2°C for *A. jeffersonianum*; Stauffer, Gates & Goodfellow, 1983). In the context of co-occurring *Ambystoma* species, the thermal optima of *A. texanum* and *A. jeffersonianum* are more similar to one another than to a commonly used outgroup that does not participate in the unisexual reproduction complex (34.6°C for *A. maculatum*; Stauffer *et al.*, 1983). This similarity between *A. texanum* and *A. jeffersonianum* suggests that any effect of thermal preference would have a small effect on higher rate of tissue regeneration in unisexuals compared to *A. texanum* and could likely not account for the large difference (36%) in regeneration rate. Overall, I agree with Werner (2016) that small differences in thermal optimum are unlikely to explain overall differences in regeneration rates.

Current measurements of species-specific thermal preferences are potentially inaccurate

Werner provides many examples to highlight the range of thermal preferences among amphibians, but these measurements are subject to a variety of factors that can influence thermal preference, such as behaviour, adaptive context, and location (Hutchison & Dupré, 1992). For example, *A. texanum* thermal preference as measured by Dupré & Petranka (1985) was based on 10 individuals from two counties in central Kentucky. Generalizing this measurement to an entire species is problematic due to the fact that amphibian thermal preferences can vary across even local populations (Freidenburg & Skelly, 2004). If thermal preferences vary in the scale of 1 km between sites across a gradient of canopy cover (Freidenburg & Skelly, 2004), then the distribution of *A. texanum*, from southern Texas to north-eastern Ohio (Petranka, 1998), likely provides a range of thermal preferences not measured by Dupré & Petranka (1985). Including biologically relevant thermal optima in comparative studies depends on the accuracy of established estimates for species or populations, and this generation of this data for amphibians lags behind other ectotherms.

Thermal preferences of polyploids may not reflect thermal preferences of parental species

How the thermal preferences of polyploid individuals relate to those of their parental species is not well understood. Werner (2016) suggests that the thermal preferences of unisexual individuals are simple additive functions of the thermal properties of the parental species that represent the majority of their subgenomes, *A. jeffersonianum*. However, this assumption may not be valid since other polyploids can display a broader range of thermal preferences than a sympatric parental species (the brine shrimp *Artemia parthenogenetica*; Zhang & Lefcort, 1991). I have no information on how thermal preferences might vary in unisexual *Ambystoma* or how they relate to the identity of the subgenomes that make up a unisexual biotype, and so it remains unknown as to how differences in species-specific thermal preferences could impact tissue regeneration in unisexuals.

Finally, Werner (2016) claims that the correlation between genome size and regeneration rate documented by Saccucci

et al. (2016) is 'not statistically significant.' I disagree, since the statistical approach we used followed standard methods for comparing rates between two groups. I interpret Werner's (2016) comments as a caution toward generalizing our findings beyond the sexual species and unisexual biotypes we studied. This could be done by assessing if the same result applies broadly among a diversity of *Ambystoma* species and unisexual biotypes using comparative framework similar to that outlined by Garland & Adolph (1994).

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