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Source: Folia Zoologica, 61(3–4) : 197-201

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: https://doi.org/10.25225/fozo.v61.i3.a3.2012

Folia Zool. – 61 (3–4): 197–201 (2012)

Metabolic costs of hybridization in newts

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Received 4 January 2012; Accepted 15 March 2012

Abstract. Heterospecific hybrids often suffer from a lowered fitness relative to parental species. Context-dependent intrinsic costs of hybridization are partially due to a malfunction in cell biochemical machinery that affects metabolic rates at the organismal level. This study examines whether heterospecific hybridization influences the metabolic costs of maintenance in F1 hybrids between closely related newts, *Triturus carnifex* and *T. dobrogicus*. When controlled for body size, oxygen consumption in hybrid newts was 59-76 % higher than in the parental species. This suggests that high standard metabolic rates in hybrids may contribute to the costs of hybridization in newts.

Key words: oxygen consumption, energetic metabolism, hybrids, *Triturus*, amphibians, mitonuclear interactions, reproductive barriers

Introduction

Heterospecific hybridization often produces phenotypes that are inferior to those of parental species. The costs of hybridization can be divided into intrinsic and extrinsic (ecological) categories. Intrinsic costs are mediated by factors, such as ploidy levels, chromosomal rearrangements, genic incompatibilities, and mitonuclear interactions that affect hybrid sterility and viability directly, i.e. independently of environment (Burke & Arnold 2001, Coyne & Orr 2004, Wolf et al. 2010). On the other hand, ecological costs can result from phenotype-environment mismatch that eliminates poorly fit but viable hybrids from parental habitats (reviewed by Schluter 2000, Coyne & Orr 2004, Rundle & Nosil 2005). In some cases, however, intrinsic costs can be context-dependent (Rundle & Whitlock 2001), which complicates discrimination between both categories.

A typical example of intrinsic costs of hybridization modulated by environmental conditions stems from mitonuclear interactions. The mitonuclear mismatch in hybrids may cause serious malfunction of metabolic machinery that affects not only fertility and viability (Breeuwer & Werren 1995, Edmands & Burton 1999, Ellison & Burton 2008) but also the metabolic costs of maintenance (Arnqvist et al. 2010, Olson et al. 2010). The influence of these costs on fitness seems highly context-dependent (Clarke 1993, Careau et al. 2008, Burton et al. 2011). If resources are readily available, selection should favour individuals with a high standard metabolic rate (SMR). However, under limited resource availability, high SMR is disadvantageous because more energy for maintenance is spent at the expense of energy that could be used for growth and reproduction. Hence, information about metabolic costs of hybridization allows formulations of testable hypotheses about hybrid 'success' in a stochastically varying environment.

The present paper examines SMR in newt species, *Triturus carnifex* and *T. dobrogicus*, and their F1 hybrids to evaluate the potential contribution of hybrid energetics to the ecological costs of hybridization between these species. Both taxa belong to the big crested newt species group in which members frequently hybridize at contact zones of their parapatric distributions (Wallis & Arntzen 1989). Genetic analyzes showed that newt heterospecific hybridization causes mitonuclear mismatch (Maletzky et al. 2008, Arntzen et al. 2009), which may affect metabolic rates, and ultimately fitness of hybrid individuals (see above). Since mitonuclear mismatch increases SMR in other taxa (Arnqvist et al. 2010, Olson et al. 2010), I predict that heterospecific hybridization increases energetic costs of maintenance in newts.

Material and Methods

Triturus carnifex (Laurenti, 1768) is a stout-bodied newt with a total length (TL) of up to 220 mm. It is distributed from Austria to southern Italy and the west Balkans at altitudes of up to 2000 m a.s.l. *Triturus dobrogicus* (Kiritzescu, 1903) is a slender-bodied elongated species with TL of up to 180 mm. The geographical range of *T. dobrogicus* is confined to the Danube River basin and several tributaries. Altitudinally it is restricted between 150 and 300 m a.s.l (see Arntzen 2003 for further information). Although both species splitted about 9 MY ago (Wielstra & Arntzen 2011), they frequently hybridize at their contact zone (Wallis & Arntzen 1989, Horák 2007).

To eliminate factors (e.g. age differences, previous thermal history) masking the presumed influence of mitonuclear interactions on standard metabolic rates, I used laboratory-bred individuals. Newts represented a F1 generation of pure *T. carnifex* (Matena, Slovenia) and *T. dobrogicus* (Veškovce, Slovakia), and *T. carnifex* (female) × *T. dobrogicus* (male) hybrids. Genetic analyses proved no mtDNA or nDNA introgression from other newt species in their original populations (Horák 2007). Details of breeding design and larval development conditions were published elsewhere (Vinšálková & Gvoždík 2007). Metamorphosed individuals of the same age (hatched in 2001) were kept in pairs (five pairs per cross) in aquaria that contained clumps of aquatic vegetation and a piece of styrofoam to allow newt emergence from water. Aquaria were placed in a temperature-controlled room. Newts were exposed to diel temperature fluctuations (14-22 °C) from April till November and as low as to 6 °C during overwintering. Light regime mimicked natural seasonal cycles. Newts were fed with live chironomid larvae, *Tubifex* worms, and zooplankton once or twice per week.

I measured SMR as O_2 consumption using push mode flow-through respirometry following a recent protocol (Kristín & Gvoždík 2012). Incurrent air was scrubbed for water and CO_2 (sodalime-silica gel and Drierite-Ascarite-Drierite scrubbers). Constant air flow (60 ± 0.1 ml min^{-1}) was generated using a mass flow-controlled pump (FoxBox-C, Sable Systems, Las Vegas, USA). Air was then rehumidified (96-98 %) using Nafion tubing (ME Series, Perma Pure, Toms River, USA) submerged in distilled water (18 ± 0.5 °C). Air humidification prevented excess evaporative water loss (< 1 % body mass; L. Gvoždík, unpublished data) in newts during a respirometry trial. Humid air entered into a baselining unit that switched between baseline and excurrent air from a multiplexer (RM-8, Sable Systems). The programmed multiplexer switched air flow among four respirometry chambers (60 ml) containing experimental animals. Air from the baselining unit entered the gas analyzers as follows: water vapor analyzer (RH-300, Sable Systems), Nafion dryer (MD Series, Perma Pure), sodalime-silica gel-Drierite scrubber, and O_2 analyzer (FoxBox-C). Analogue outputs from gas analyzers were connected to a high-resolution analogue to digital converter (UI-2, Sable Systems) which sent data to the PC. The second flow circuit pushed the rehumidified air at the same flow rate into multiplexer to reduce hypoxic conditions or accumulation of CO_2 in closed chambers. The flow rate through the system was verified by measuring excurrent flow rates using a calibrated mass flow meter (FlowBar-8, Sable Systems). The oxygen analyzer was calibrated before each trial using H_2O- and CO_2-scrubbed air.

Newts starved for seven days prior to respirometry measurements to avoid a confounding effect of specific dynamic action (Feder et al. 1984). After weighing (to 0.01 g), each individual was placed into a respirometry chamber that was placed in water bath maintained at 18 ± 0.5 °C. This temperature falls within the range of preferred body temperatures in both parental species (Gvoždík 2003, 2005). The room temperature was kept at 22 °C to avoid water condensation inside the system. The respirometry data were recorded at 1 Hz using ExpeData software (Sable Systems). The baseline and multiplexer was programmed so that each newt was continuously measured for 675 s per hour. Baseline (180 s) was taken before and after each measured interval. Newt spontaneous activity was monitored using four cameras (5 fps) connected to a digital surveillance system (Chateau Corp., Taiwan). Simple motion activity detection recorded the numbers of newt movements in 10 s intervals during the whole trial (six hours). This was sufficient to rule out the possibility that minimal values of oxygen consumption (see below) were affected by locomotor activity. Since newts are predominantly nocturnal, one or two trials per day were realized during their typical inactivity period (8:00-14:00 and 15:00-21:00) between the 15th-19th of August, 2011. Newts occurred on both land and water during this period, and thus it seems likely that the influence of their prolonged exposure to terrestrial conditions had negligible effect on SMR measurements.

Raw O_2 measurements were drift corrected (fourth polynomials) prior to further analyses. Oxygen consumption ($\dot{V}O_2$; ml h^{-1}) was calculated as $\dot{V}O_2$ =

FR $(F_iO_2 - F_eO_2)/(1 - F_eO_2)$ where FR is flow rate, F_eO_2 is fractional concentration of excurrent O_2, and F_iO_2 is a fractional concentration of incurrent O_2. Standard metabolic rate was estimated as the lowest 90 s moving average in each individual. The influence of hybridization on O_2 consumption was tested using the analysis of covariance with body mass and activity during measured interval as the covariates. Continuous variables ($\dot{V}O_2$ and body mass) were log transformed and activity counts were square root transformed before the analysis. For a "cross" factor, two a priori orthogonal contrasts were specified: hybrids vs. parental species and *T. carnifex* vs. *T. dobrogicus*. The deletion test was applied to find the minimum adequate model (Crawley 2007). All analyses were performed using library "MASS" in R (R Development Core Team 2009).

Results

Due to increased mortality of overwintered newts in 2008 and marked drop in body mass in one individual, the original sample size was reduced from 30 individuals to 19 (*T. carnifex*: $n = 7$; *T. dobrogicus*: $n = 3$; hybrids: $n = 9$). Adding the interaction between cross and body mass provided no improvement for the overall model fit ($F_{2,12} = 0.47$, $P = 0.64$), and thus the simple additive model was used for statistical inference. Mean locomotor activity during measured intervals was low (2–3 movements) and similar among crosses ($z = 0.234$, $P = 0.81$). Given the negligible contribution of activity to the overall model fit ($F_{1,14} = 1.61$, $P = 0.23$), this factor was also omitted from the minimum adequate model. Newt oxygen consumption was influenced by both cross combination and body mass (cross: $F_{2,15} = 7.32$, $P = 0.006$; body mass: $F_{1,15} = 12.04$, $P < 0.001$; Fig. 1). Specifically, hybrid oxygen consumption was higher (0.673 ± 0.053 ml h^{-1}) than in both parental species ($t_{15} = 2.78$, $P = 0.01$), which showed similar body mass-adjusted values (*T. carnifex*: 0.428 ± 0.061 ml h^{-1}; *T. dobrogicus*: 0.381 ± 0.094 ml h^{-1}; $t_{15} = 0.66$, $P = 0.52$). The mass-specific metabolic rates that are frequently used for comparative analyses were as follows: *T. carnifex*: 0.034 ± 0.005 ml g^{-1} h^{-1}; *T. dobrogicus*: 0.027 ± 0.007 ml g^{-1} h^{-1}; hybrids: 0.053 ± 0.004 ml g^{-1} h^{-1}.

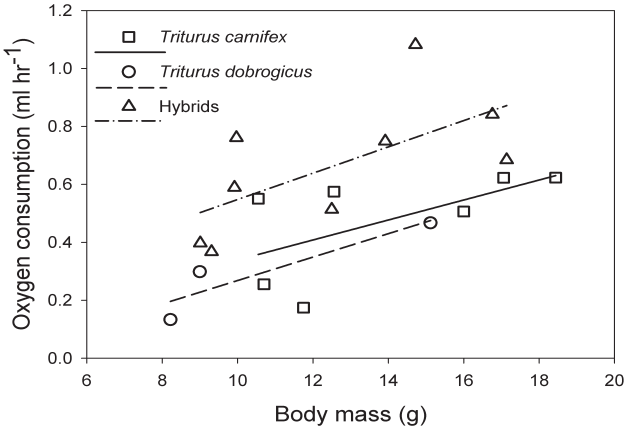

Fig. 1. *Minimum oxygen consumption at 18 °C as a function of body mass in newts,* Triturus carnifex*,* T. dobrogicus*, and their F1 hybrids. Data were fitted using least-squares linear regression.*

Discussion

In this study, hybrid newts spent markedly more energy for maintenance than the parental species. This concurs with findings in other taxa, in which mitonuclear mismatch increased standard metabolic rates (Arnqvist et al. 2010, Olson et al. 2010). Although limited sample size and set of laboratory conditions preclude strong inference from the results here presented, they provide some interesting implications for further research.

Since hybridization produces mitonuclear mismatch in newt hybrids, it seems likely that high standard metabolic rates resulted from this genetic interaction. Previous results showed that mitonuclear incompatibilities reduce mitochondrial ATP production (Yamaoka et al. 2000, Ellison et al. 2008). Accordingly, this may lead to increased oxygen consumption at the organismal level to compensate for the lowered ATP production (Olson et al. 2010). Poor mitochondrial respiratory function may produce a higher amount of oxygen-free radicals that trigger energetically costly mtDNA transcription and expression of new respiratory complexes (Lane 2011), and thereby contribute further to maintenance costs in hybrids. Alternatively, higher SMR in hybrids may reflect their higher genetically-determined mitochondrial density within cells (Arnqvist et al. 2010). Discriminating among various proximate causes underlying energetic costs in hybrids will provide interesting research plan for further studies.

As noted above, the putative influence of high hybrid SMR on fitness depends on the relative availability of resources in a given habitat. In newts, several lines of evidence suggest that high SMR are detrimental to hybrids. First, newt terrestrial activity is greatly restricted by temperature and moisture availability (Jakob et al. 2003), and thus a high SMR spent excess energy that could be invested to fitness-enhancing activities. In addition, interspecific comparisons showed that SMR in newts and salamanders are lower

than in frogs (Gatten et al. 1992), which indicates a general trend towards the very economical lifestyle of this group. Secondly, during the aquatic phase, high oxygen consumption increases diving frequency which consequently should affect reproductive success (Halliday & Sweatman 1976) and predation risk (Kramer 1988) of hybrids especially in warmer environment (Šamajová & Gvoždík 2009). Finally, if the pronounced differences in SMR persist at low temperatures during wintering, the inactivity period will be more costly for hybrids than for parental species. Hence, from the energetic view, the persistence of newt hybrids in a given habitat will depend on both the availability of resources and abiotic limitations (temperature, moisture) of their activity time.

In conclusion, the present study provided metabolic data from rarely measured European newts to promote an "energetic" view on the costs of hybridization. Despite the intrinsic origin of metabolic costs, the link between energetic metabolisms and life-history traits is obvious (Brown et al. 2004), and thus it seems likely that high hybrid SMR contribute also to the ecologically-mediated hybrid inferiority. Unfortunately, information about energetic costs of hybridization is lacking from previous morphology-based studies on extrinsic postzygotic barriers, and so it remains unclear to what extent the sole emphasis on intermediacy of hybrid phenotypes has been exaggerated. Hence, the focus on whole-animal metabolic measurements in connection with selective importance of mtDNA and mitonuclear interactions (Rand 2001, Ballard & Whitlock 2004, Dowling et al. 2008) provides a promising tool for further studies.

Acknowledgements

This study is a tribute to great Czech zoologist J. Zima on the occasion of his 60th birthday. The Czech Science Foundation (P506/10/2170), the Czech Ministry of Education (LC06073), and institutional support (RVO:68081766) provided funding for this study.

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