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From population ecology to metabolism: growth of *Trypanosoma evansi*, and implications of glucose depletion, in a live host

S. Andrea Moreno [a, *], Jesús Molinari [a], Mayerly Nava [b]

[a] Departamento de Biología, Facultad de Ciencias, Universidad de Los Andes, Mérida 05101, Estado Mérida, Venezuela
[b] Departamento de Biología, Facultad Experimental de Ciencias, Universidad del Zulia, Maracaibo, Estado Zulia, Venezuela

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ABSTRACT

Little attention has been paid to the potential top-down effects of population ecology on metabolism. Because it is capable of a fast growth that dramatically modifies its blood environment, the parasitic protozoan, *Trypanosoma evansi*, is a promising model for the study of density-dependent metabolic processes. We assess the *in vivo* growth rate, doubling time, biomass yield, glycolytic flux, and enzyme expression of *T. evansi*. Then, we explore the metabolic changes likely occurring during its growth. At low *T. evansi* densities, most host glucose is used to produce energy, which results in the release of pyruvate. Part of glucose is used for NADPH production through the pentose phosphate pathway. At high *T. evansi* densities, fructose, mannose, and glycerol become additional energy sources. Part of host glucose is used for biosynthesis and for NADPH production through alternative metabolic pathways, which results in the release of succinate, alanine, and acetate. The ability of organisms to adjust to resource changes is crucial to their survival. Irrespective if the triggering mechanism is direct (nutrient limitation) or indirect (a pheromone-like factor), nutrient availability is necessarily the main evolutionary factor responsible for the density-dependent metabolic properties of trypanosome populations.

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1. Introduction

The haemoparasitic protozoan, *Trypanosoma evansi*, is polyphyletically originated from its antecessor, *Trypanosoma brucei brucei*, from which it differs genetically only slightly. Therefore, its status as a separate species, or even subspecies, is questionable (Lai et al., 2008; Carnes et al., 2015). *T. evansi* should probably be referred to as dyskinetoplastic *T. brucei brucei*, or as *evansi*-type *T. brucei brucei* strains, but such nomenclature is yet to be established. *T. evansi* causes surra and *T. brucei brucei* causes nagana, two similar diseases affecting nonhuman mammals. Surra is transmitted by several genera of hematophagous flies occurring throughout the tropics and subtropics (Desquesnes et al., 2013). Nagana is transmitted exclusively by the endemic sub-Saharan tsetse flies (Lun and Desser, 1995). A complete loss of kinetoplast maxicircles, a genetic material required for transformation and multiplication within tsetse flies, differentiates *T. evansi* from *T. b. brucei* (Lun and Desser, 1995; Lai et al., 2008). As a consequence, *T. evansi* is monomorphic, existing only as a bloodstream form (BSF) transmitted

* Corresponding author.
E-mail addresses: sandrea@ula.ve, sandreamoren@gmail.com (S.A. Moreno).

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mechanically (Lun and Desser, 1995; Desquesnes et al., 2013). In contrast, *T. b. brucei* is polymorphic, existing as a BSF in mammals, and as a procyclic form in tsetse flies. The capability to use cosmopolitan vectors may have enabled *T. evansi* to spread outside Africa (Lun and Desser, 1995).

Biochemistry is to ecology what genetics is to evolution: the ecology of populations, communities and ecosystems is determined by the biochemical processes of component organisms (Brown et al., 2004). By participating in up to 75% of food web links, parasites, which in turn depend on their own metabolism, have a major impact on the properties and health of ecosystems (Hudson et al., 2006; Lafferty et al., 2006). Although the bottom-up (i.e., for populations, and subsequently for communities and ecosystems) relevance of the metabolism of individual organisms has received considerable attention (Brown et al., 2004), the converse is not true: few studies (e.g., Sharma et al., 1979; Zaman et al., 2008; DeLong and Hanson, 2009; Schwede et al., 2012) have addressed the problem of how density and other population parameters exert a top-down influence on the metabolism of individual organisms.

Monomorphic strains of African trypanosomes, such as *T. evansi*, are extremely virulent: inoculation of a single trypanosome may lead in few days to a host parasite burden in excess of 10^9 trypanosomes/ml (Seed and Wenck, 2003). In the course of such a steep population increase, these trypanosomes dramatically modify their blood environment (Hörchner et al., 1983; Seed and Wenck, 2003; Abdelrahman et al., 2004). Therefore, monomorphic African trypanosomes may represent promising model organisms for investigating the effect of density-dependent environmental changes on the metabolic processes of growing populations. For BSF African trypanosomes, little *in vivo* information exists on the growth rate, doubling time, glycolytic flux, biomass yield, and density-dependent enzyme expression. In this study, we provide this kind of data for *T. evansi* growing in rat bloodstream. We explore the metabolic changes likely involved in the interaction of growing populations of *T. evansi*, and other trypanosomes of the subgenus *Trypanozoon*, with blood levels of glucose and other nutrients.

2. Materials and methods

In the case of abbreviations, explanations not found in text appear in Table 1. Laboratory procedures complied with Venezuelan laws, and with the EU Directive 2010/63/EU for animal experiments.

Our stock of *Trypanosoma evansi* (TeAp-N/D1, Perrone et al., 2009; previously known as TEVA1, Espinoza et al., 1997) comes from naturally infected horses in the Venezuelan Llanos region, where equine trypanosomiasis is endemic (Moreno et al., 2013). We cultured parasites in adult (body masses ~240 g) female Sprague–Dawley rats. For the culture of parasites, and in all experiments involving infected rats, we inoculated each animal intraperitoneally with 100 μl of infected rat blood harboring live *T. evansi* at a concentration of 3.6×10^8 parasites/ml. We provided infected rats with rodent lab blocks and water *ad libitum*. To maintain live *T. evansi* in liquid nitrogen for long periods of time, we used cryovials containing phosphate buffer saline with 1% glucose (PBSG) and 5% dimethyl sulphoxide (DMSO).

2.1. Population growth within a mammalian host

We used 16 infected rats. We divided randomly the animals into four equally sized groups, which we sacrificed sequentially at intervals of 24, 36, 48, and 60 h. We diluted the blood sample from each sacrificed animal 200–400 times in a 300 mOsm NaCl solution containing 0.5% formaldehyde. We used a Neubauer haemocitometer to estimate the

Table 1
Specific activities (μmol × min^{-1} × mg^{-1}) and relative signal of four enzymes in subcellular fractions, and relative signals of two enzymes in intact cells during the population growth of *T. evansi*. Plain numbers indicate specific activities. Bracketed numbers indicate relative (% of maximum number of pixels) intensities of signals visible in western blots. Highest values in bold.

	Hexokinase (HK)	Phosphoenolpyruvate carboxykinase (PEPCK)[a]	Enolase (ENO)[a]	Malic enzyme (ME)[a]	%
Subcellular fractions: differential centrifugation					
Homogenate (H)	1.18 [40.79]	7.00 [41.60]	90.00	36.00	100.00
Nuclear fraction	0.14 [31.76]	2.65 [47.07]	0.50	6.30	17.24
Large granular fraction	2.97 [43.22]	10.25 [50.84]	0.23	17.30	11.13
Glycosome-rich fraction	**4.44 [93.14]**	**61.85 [100.00]**	0.14	10.47	7.83
Microsomal fraction	1.98 [38.31]	8.00 [26.23]	5.00	9.81	11.70
Cytosolic fraction	0.05 [9.89]	2.69 [2.62]	**124.00**	**84.40**	52.10
Subcellular fractions: isopycnic ultracentrifugation					
Purified glycosomes (PG)	**7.91 [100.00]**	**118.00 [99.43]**	0.00	0.00	–
Purification factor (PG/H)	6.70 [2.45]	16.86 [2.39]	–	–	–
Intact cells: relative enzyme signal during growth[b]					
7.2×10^7 parasites/ml	**[100.00]**	[47.46]	–	–	–
1.8×10^8 parasites/ml	[84.72]	**[100.00]**	–	–	–
5.0×10^8 parasites/ml	[90.77]	[80.00]	–	–	–
2.0×10^9 parasites/ml	[92.25]	[73.19]	–	–	–

% = proportion of total protein in each fraction with respect to homogenate.
[a] Specific activity values must be multiplied ($\times 10^{-3}$).
[b] Signals of HK and PEPCK are negatively correlated to each other ($r = -0.999$; $p < 0.01$).

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