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The role of metabolism in understanding the altitudinal segregation pattern of two potentially interacting lizards

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ABSTRACT

Sympatric species from the same ecological guild, that exhibit partial altitudinal segregation, can potentially interact in areas of syntopic occurrence. Besides general species' ecology, physiology can provide important answers about species interactions reflected in altitudinal patterns. Lizards *Podarcis muralis* and *Iberolacerta horvathi* exhibit partial altitudinal segregation, while they strongly resemble in overall morphology and ecology (diet, daily and seasonal activity pattern), but show certain degree of physiological dissimilarity. They have similar mean preferred body temperatures and patterns of seasonal and daily variations but differ in the magnitude of seasonal variation. Since an ectotherm metabolism is highly dependent on body temperature, thermoregulation is expected to directly affect their metabolism. We compared metabolic rates of adult males from an area of sympatry, measured under two temperature regimes (20 °C and 28 °C). Both species increased metabolic rates with temperature in a similar pattern. We also compared electron transport activity from tail tissues which provide values of species' potential metabolic activity (enzymatic capacity). Species clearly differed in potential metabolic activity; *I. horvathi* attained higher values than *P. muralis*. No difference was detected in how species exploited this potential (calculated from the ratio of electron transport activity and metabolic rates). However, we observed higher potential metabolic activity *I. horvathi* which together with the ability to thermoregulate more precisely could represent a higher competitive advantage over *P. muralis* in thermally more restrictive environments such as higher altitudes. Understanding of metabolism seems to provide valuable information for understanding recent distributional patterns as well as species interactions.

1. Introduction

Sympatric species from the same ecological guild are likely to be in interaction when in areas of syntopic occurrence (Begon et al., 2006). One of the common distributional patterns of interacting species is partial altitudinal segregation, where one species is occupying higher altitudes, the other lowlands and there is a zone of spatial overlap at intermediate altitudes (e.g. Vrezec and Tome 2004, McHugh and Budy 2005, Pasch et al. 2013, Žagar et al. 2013). Such sympatric zones of overlap provide ideal opportunity to study species interactions.

The first step of species interaction studies is usually a comparison of species' general ecological traits, and only rarely physiology is included although it can provide elusive answers. Especially metabolism could be an important physiological trait providing a better understanding of species distribution patterns and interactions (e.g. Bennet et al., 1984; Nagy et al., 1984). From a number of factors that may influence the metabolic rate (MR) of reptiles, the most dramatic effects are probably those produced by changes in the activity level mediated by body temperature (Bennett and Dawson, 1976; Huey, 1982). However, within a species, body size, sex, reproductive and nutritional conditions, time of day and seasonal acclimatization also affect the metabolic rate (Bennett, 1982; Patterson and Davies, 1984; Brown et al., 1992; Zari, 1996, 1999; Dorcas et al., 2004; Steffen and Appel, 2012). Also, different species that share similar habitats may display divergent metabolic rates even as a result of separate evolutionary history (Thompson and Withers, 1994; Randriamahazo and Aime, 1998; Hare et al., 2006).

The sympatric lacertid lizards from the Northern Dinaric Mountains, Common Wall Lizard (*Podarcis muralis* Laurenti, 1768) and Horvath's Rock Lizard (*Iberolacerta horvathi* Méhely, 1904), exhibit partial altitudinal segregation pattern (Žagar et al., 2013). *I. horvathi* is most abundant at higher altitudes while *P. muralis* in lowlands and a broad zone of overlap exists at mid-altitudes. A specific characteristic of the overlapping area at mid-altitudes in SE Europe is high forest cover (Perko and Orožen Adamič, 1998) where open areas, suitable for reptile occurrence

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and basking, are limited (Žagar et al., 2013). Males of many species of the family Lacertidae are known to exhibit aggressiveness against other males in spatial context, namely for the best basking places and shelters (e.g. Olsson, 1994; López and Martín, 2002). Thus, while such favourable parts of the habitat are limited in the area of sympatric occurrence of the studied species, there is a high potential for interactions.

Both species strongly resemble in morphology (Žagar et al., 2012) and ecology (diet, daily and seasonal activity pattern and reproduction, De Luca, 1989; Lapini et al., 1993; Richard and Lapini, 1993; Cabela et al., 2007), but show some degree of physiological dissimilarity. Osojnik et al. (2013) recently described that both species have similar mean preferred body temperatures (T_p) and the pattern of daily and seasonal variations of T_p. The only interspecific difference detected was the magnitude of seasonal variation of T_p; the "high altitude" species, *I. horvathi*, selected similar temperatures throughout the year, while the "low altitude" species, *P. muralis*, displayed seasonal variation. This suggests that *I. horvathi* is potentially a more precise thermoregulator than *P. muralis*. Alongside, *I. horvathi* was found to lose less water than *P. muralis* when exposed to dry conditions, suggesting additional physiological trait enabling it to spend more time in sun basking without risking water stress (Osojnik et al., 2013).

Since acclimatization of preferred body temperature (T_p) in response to environmental seasonality has been demonstrated to vary between lizard species (e.g. Angilletta, 2001; Osojnik et al., 2013) and temperature is the key determinant of metabolic rate in ectotherms (Bennett, 1982), difference in metabolism between species is expected. As a general rule in ectotherms, species from cold climates tend to have a higher metabolic rate at low temperatures than those from warm climates. For example, the cold-climate lizard *Zootoca vivipara* attained higher metabolic rates than the warmer-climate lizard *Podarcis hispanica* (Patterson and Davies, 1989). With altitude, the most obvious changing environmental trait is the temperature (dropping with increase of altitudes) which has a pronounce direct effect on ectotherms, such as lizards (Huey et al., 2012).

Organisms' metabolism is constraint by the enzymatic capacity of tissues. This can be measured in several ways, most often by assessing citrate synthase activity or cytochrome *c* oxidase (e.g. Simon and Robin, 1971; Lannig et al., 2003; Seebacher et al., 2003), whereas electron transport system (ETS) activity was less frequently used (e.g. Lannig et al., 2003) but provides similar information. Measurements of ETS activity indicate the amount of oxygen consumption that would occur if multi-enzyme complexes, located in the inner membrane of mitochondria, functioned maximally (Muskó et al., 1995). The ratio between ETS activity and observed oxygen consumption (ETS/MR ratio) is an important index of an organism's metabolism (Muskó et al., 1995) because it provides a measure of the fraction of total metabolic potential that is actually exploited for respiration. Several studies also calculated the ETS/MR ratio (but only in invertebrates) and reported that it differs between species having different ecological demands and consequently inhabiting different habitats (Muskó et al., 1995; Simčič and Brancelj, 1997; Simčič et al., 2005). Measuring metabolic potential provides information on species' fundamental physiology and is therefore crucial for a comprehensive understanding of an organisms' metabolism.

Our study was set to explore the metabolic respiration, the response of metabolism to temperature change and to determine metabolic potential, which was compared between studied species to elucidate the underlying distributional pattern (partial altitudinal segregation) and the potential for interaction in syntopic populations. We measured metabolic rate (oxygen consumption) and determined metabolic potential (ETS activity) under two realistic temperature regimes: at 20 °C, which is a less favourable temperature for lizard activity, and at 28 °C, the temperature close to their T_p (Osojnik et al., 2013). If both species were adjusted to their current altitudinal ranges, the metabolic rates and potential should be higher in the mountain species (*I. horvathi*) than in the lowland one (*P. muralis*), while the use of metabolic potential (calculated from the ratio of metabolic rate and metabolic potential at the same temperature) should be lower for the mountain species ensuring a reserve for thermally critical periods. Furthermore, metabolic response to temperature was expected to differ; species with a lower precision of thermoregulation (*P. muralis*) was expected to have higher metabolic rate at lower temperatures as a consequence of compensation for attaining lower T_p in spring, when the thermal environment is more restrictive (Osojnik et al., 2013). Obtained results were linked to species ecology to provide a better understanding of potential interspecific interaction.

2. Material and methods

2.1. Field study sites and lizard collection

Lizards were collected in the area of species' sympatric occurrence (Kočevje, SE Slovenia: lat. 45°38′N, long. 14°51′E, datum = WGS8410). To discard effects of ontogeny or egg production only adult male lizards were collected. Collecting sites were similar in habitat structure and altitude (*P. muralis* site: 723 m a.s.l., and *I. horvathi* site: 986 m a.s.l.), and were located at a distance of 9.4 km one from another. Previously seasonal differences in enzymatic activity have been detected in several terrestrial species of lizards (e.g. Garland and Else, 1987; Zari, 1996, 1999), thus to avoid possible effect of season, all collections were conducted in the summer in three sampling campaigns between 26th June and 15th August 2013. Live lizards were quickly transported to the laboratory in the dark in order to reduce stress effects. Before the experiments, lizards were kept in individual housing terraria for three to five days with food (*Tenebrio molitor* larvae) and water provided ad libitum, and under a natural regime of light and temperature maintained by switching infra-red bulbs on for the period of 8 am to 18 pm (see also Osojnik et al., 2013). Food supply was interrupted two days previous to the experiment.

2.2. Collection permit and ethical procedures

All individuals used in the experiments were collected under the special licences 35601-14/2013-5 issued by the Slovenian Environment Agency. All animal handling procedures were done following the EU and Slovenian Government-established norms and procedures.

2.3. MR and ETS activity

Metabolic rate (MR, as a measure of oxygen consumption) was measured consecutively at two temperature regimes (20 °C and 28 °C). The experiment was performed in randomised trials from 08.30 to 16.30 h Central European Time (CET), the period of daily activity of both species observed in the field (Brana, 1991; De Luca, 1992). Lizards were put in 200 mL individual glass flasks with an impermeable lid and MR was measured with a fibre-optic oxygen meter (PreSens, Regensburg, Germany), each lizard individually in one flask. In the first part of the experiment, the flasks with lizards were first kept for 20 min at 20 °C to acclimatize. Then we measured the oxygen concentration at hourly intervals for four consecutive times, each time in the same order starting from flask number one, continuing until the last number. Each measuring session was done in less than three minutes to minimise disturbance of lizards during the experiment. After this, flasks were opened, with lizards remained kept inside, and moved to 28 °C where lizards were left for 20 min to heat-up. After that we closed the flasks and left it for an additional 20 min after starting the first measurement. Again we measured the oxygen concentration at hourly intervals for four consecutive times. In each series we used 18–28 flasks, depending on the number of individuals used per session. In all cases two of the flasks were left empty and represented controls. Oxygen consumption by each lizard was determined as the difference between oxygen consumption in the flask with the animal and mean oxygen consumption in control flasks. Oxygen consumption in each flask was calculated as the