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Discovering divergence in the thermal physiology of intertidal crabs along latitudinal gradients using an integrated approach with machine learning

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ABSTRACT

In intertidal marine crustaceans, phenotypic variation in physiological and life-history traits is pervasive along latitudinal clines. However, organisms have complex phenotypes, and their traits do not vary independently but rather interact differentially between them, effect that is caused by genetic and/or environmental forces. We evaluated the geographic variation in phenotypic integration of three marine crab species that inhabit different vertical thermal microhabitats of the intertidal zone. We studied seven populations of each species along a latitudinal gradient that spans more than 3000 km of the Chilean coast. Specifically we measured nine physiological traits that are highly related to thermal physiology. Of the nine traits, we selected four that contributed significantly to the observed geographical variation among populations; this variation was then evaluated using mixed linear models and an integrative approach employing machine learning. The results indicate that patterns of physiological variation depend on species vertical microhabitat, which may be subject to chronic or acute environmental variation. The species that inhabit the high- intertidal sites (i.e., exposed to chronic variation) better tolerated thermal stress compared with populations that inhabit the lower intertidal. While those in the low-intertidal only face conditions of acute thermal variation, using to a greater extent the plasticity to face these events. Our main results reflect that (1) species that inhabit the high-intertidal maintain a greater integration between their physiological traits and present lower plasticity than those that inhabit the low-intertidal. (2) Inverse relationship that exists between phenotypic plasticity and phenotypic integration of the physiological traits identified, which could help optimize energy resources. In general, the study of multiple physiological traits provides a more accurate picture of how the thermal traits of organisms vary along temperature gradients especially when exposed to conditions close to tolerance limits.

1. Introduction

Physiological patterns that characterize different populations are strongly defined by environmental conditions (Hoffmann and Parsons, 1989; Somero, 2002; Khaliq et al., 2014) that, among others, determine distribution ranges, tolerance capacities, and ultimately organismal fitness. One of the main abiotic factors that affects physiological changes in ectotherm organisms is temperature (Johnston and Bennett, 2008; Castañeda et al., 2005; Mora and Maya, 2006; Angilletta, 2009; Lardies et al., 2011). Specifically, temperature has been shown to influence basic organismal functions, biochemical rates, locomotion, growth and reproduction (Kingsolver and Huey, 2008; Somero, 2010; Gaitán-Espitia et al., 2013a, 2013b, 2014). Therefore, temperature plays a fundamental role in species distribution patterns (Somero, 2005; Deutsch et al., 2008; Calosi et al., 2008). Latitudinal gradients along with intertidal gradients provide natural variation that can be used to investigate how temperature affects thermal physiology (Stillman and Somero, 2000; Helmuth et al., 2006). Coastal areas can be considered as natural laboratories where resident organisms may differ in terms of local adaptation and/or phenotypic plasticity, both mechanisms that allow populations to maximize fitness in response to environmental heterogeneity (Gardiner et al., 2010; Yampolsky et al., 2014).

Variations in physiological traits along environmental gradients are causes and consequences of phenotypic divergence in natural populations (Torres Dowdall et al., 2012), conferring local fitness advantages (Kawecki and Ebert, 2004). In general, these evaluations of phenotypic differentiation have been often correlated usually with latitude (Lindgren and Laurila, 2009; Zippay and Hofmann, 2010). Overall, The problem is that the statistics are limited due to the lack of flexibility by incorporating only univariate and linearity for estimations (Naya et al., 2011; Sunday et al., 2014; Weber et al., 2015) with a limited capacity of data interpretation. Others have shown that environmental variation affects integrated phenotypes involving several co-dependent traits (see, Salazar-Ciudad, 2007; Armbruster et al., 2014). Furthermore, phenotypic integration provides an explanation for how phenotypes are sustained by relationships between traits (Pigliucci and Preston, 2004). Because physiological traits play an important role in fitness (Ricklefs and Wikelski, 2002), the environmental characterization plays an important role in the local adaptation for the effectiveness in the survival and reproduction of the populations (McLean et al., 2014). In this sense,

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understanding how environmental gradients (for example, temperature) have effects on physiological traits is desirable to understand how the increase in global temperature can affect different populations (Magozzi and Calosi, 2015). In general, measuring physiological traits in a population reflects the costs and benefits associated with somatic maintenance in thermal environments (Heusner, 1985; Clarke and Johnston, 1999; Watson et al., 2014). Metabolic rate is the main parameter used to measure subsistence energy costs being directly related thermal sensitivities (Ruel and Ayres, 1999; Kovac et al., 2014) and thermal safety (see Sunday et al., 2011). Recovery time after critical thermal events also provides an index of how sensitive species are to climate (Castañeda et al., 2004, 2005). Also, morphometric characteristics have been shown to follow some biogeographic patterns, mainly latitudinal patterns (Angilletta et al., 2004; Bidau and Martí, 2007; Zamora-Camacho et al., 2014).

Analyses involving computational intelligence could provide an understanding of the patterns that emerge from the interaction between organismal traits and how these interactions can be modified by the environment (Park and Chon, 2007). In most cases, these interactions between traits are often too complex and do not meet the assumptions of conventional statistical procedures (Recknagel, 2001; Kampichler et al., 2010). Machine learning has many applications (see Olden et al., 2008; Thessen, 2016), but notably it has been shown to be useful when disentangling associated variables to gain a deeper understanding of multiple interactions (Peters et al., 2014). In this sense, the phenotypic divergence in an integrated phenotype has been related to the relatively low amounts of phenotypic covariance in closely related populations (Game and Caley, 2006; Renaud et al., 2006) and other studies have shown otherwise (Arnold and Phillips, 1999), generally adjusting multivariate linear models. Therefore, more studies comparing the relationships between the traits are clearly necessary to understand the link in the divergence between populations. The number of methods used for integration in machine learning has grown steadily (Acevedo et al., 2009; Valletta et al., 2017). Therefore, there are multiple models that differ in the technique of integrating the variables. Overall, in order to better understand associations between multiple associated variables, a reduction in dimensionality is a key factor in the simplification of analysis (Kasun et al., 2016). Machine learning methods, in general can fall into two categories: (1) unsupervised learning (i.e., clustering), that identifies patterns in a heuristic way (Sathya and Abraham, 2013) and (2) supervised learning (i.e., classification) which can be used to infer a function from labeled training data. Due to the existence of many methods that perform similar machine learning functions, it is pertinent to compare different algorithms, since the performance of each algorithm differs given the clustering/classification problem (Caruana and Niculescu-Mizil, 2006; Übeyli, 2007), in order to unmask the patterns of association between the traits that emerge from the population divergence.

Using three crab species, which are distributed along a small vertical intertidal gradient (i.e. intertidal zone), we analyzed variation in thermal exposure at different spatial/temporal scales. Specifically, crabs in the lower intertidal experience acute thermal variation because they are exposed to periods of greater thermal changes only in periods of extremely low tides, while those in the high intertidal experience chronic thermal variation determined by daily tidal cycles. In addition, different populations of these species are distributed along a latitudinal-environmental gradient that covers more than 3000 km and is marked by gradual thermal variation (Barría et al., 2014; Gaitán-Espitia et al., 2014). We performed trait integration using machine learning, which allowed us to unravel differences that exist in the degree of association among physiological traits of crabs and their relation with phenotypic plasticity (see Gianoli and Palacio-López, 2009). To investigate the physiological divergence among these closely related organisms that inhabit different habitats, we determined the variation in the phenotypic matrix. Finally using both conventional statistics and machine learning methods, we investigated the thermal geographic variation of physiological traits in intertidal crabs to determine the variation in a complex phenotype along the latitudinal and intertidal gradients.

2. Materials and methods

2.1. Model species and intertidal variability

Samples of three species of intertidal crustaceans (i.e., *Cyclograpsus cinereus*, *Petrolisthes violaceus*, and *Petrolisthes tuberculosus*) were used for this study. Specifically, samples were collected at three different levels within the intertidal zone: High (0.6–1.0 m), Middle (0.3–0.5 m) and Low (0.1–0.2 m). The high intertidal is characterized by chronic environmental variability, the low intertidal experiences acute variability, and the middle intertidal can be considered as a transition zone. The species used here have broad latitudinal distributions: *C. cinereus* (from Ancon, Peru to Calbuco, Chile), *P. violaceus* (Callao, Peru to Peninsula de Taitao, Chile) and *P. tuberculosus* (Callao, Peru to Chiloe, Chile) (Zagal and Hermosilla, 2007). The samples were collected from seven locations along the coast of Chile: Iquique (20° 19′ 07.5″), Antofagasta (23° 46′ 30.8″), Talcaruca (30° 29′ 32.9″), El Tabo (33° 27′ 23.8″), Lenga (36° 45′ 36.6″), Playa Rosada (39° 49′ 46.4″), and Playa Brava (41° 52′ 00.4″) (see SM1; Fig. 1). The sampled locations cover almost the complete geographic range of these species in Chile (3000 km). It should be noted that within the wide latitudinal range that covered our study, two barrier with strong environmental changes could be identified: 1) biogeographic break at 30°S has been identified within the latitudinal range sampled. Specifically, the composition and abundance of biota differ on either side of this break (Navarrete and Wieters, 2000; Navarrete et al., 2014). 2) Environmental barrier of lower intensity at 36°S has also been documented in coastal zones (see Fig. 1) and has been attributed to the Neogene development of a shallow oxygen minimum zone (OMZ) (Martinez-Pardo, 1990) and wind-induced coastal upwelling. In each sample site, we monitored the temperature of the intertidal continuously using high-resolution loggers (Tidbit®, Onset Computer Corp., MA, USA). The thermal loggers used track both sea surface temperature as well as air temperature during extreme tides; thus, we recorded the environmental temperature fluctuations characterizing these sites every 30 min during three years.

The crabs were collected during spring of 2012–2013 (see SM1), and to remove possible effects of sex, only male crabs were collected and used in the physiological measurements. Covered with icepacks and placed in a cooler, sampled individuals were transported to the laboratory of evolutionary ecology at the University Adolfo Ibáñez, Santiago, Chile. Crabs were acclimated for three weeks to the following conditions: artificial seawater at 33 psu (Instant Ocean®), temperature of 14 °C, photoperiod of 12:12 light/darkness, aeration and constant feeding (Instant Algae, Shellfish Diet® and TetraFin Food Flakes®).

2.2. Physiological traits

2.2.1. Metabolic Rate 14 °C (MR14) and 20 °C (MR20)

The metabolic rate was calculated based on the oxygen consumption during a period of time (Gaitán-Espitia et al., 2014, 2017). After acclimation, each crab was incubated in artificial seawater at 14 °C and placed in 113 ml respirometric chambers. The oxygen concentration within the chamber was quantified every 15 s for a maximum of 2 h an optical sensor connected to an Oxygen register MINI-OXY-4 (PreSens, GmbH, Germany), If the concentration is reduced below 75% of the initial oxygen concentration, the incubation is over. The sensor was calibrated with synthetic seawater (saturated: 100%) and a sodium sulfite solution (0%). We determine the metabolic rates of the crab at 14 °C (mean temperature of the coast of Chile; see Gaitán-Espitía et al., 2017). Then, we acclimated the crabs to 20 °C for a period of three weeks to estimate the metabolic rate at that temperature. The 6 °C of thermal increase in our experimental treatments are consistent with the trend of the increase in the average ambient temperature expected for