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Low quality diet and challenging temperatures affect vital rates, but not thermal tolerance in a tropical insect expanding its diet to an exotic plant

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ABSTRACT

Determining responses of organisms to changing temperatures is a research priority, as global warming threatens populations and ecosystems worldwide. Upper thermal limits are frequently measured as the critical thermal maximum (CT_{max}), a quick bioassay where organisms are exposed to increasing temperatures until individuals are not able to perform basic motor activities such as walking or flying. A more informative approach to understand organism responses to global warming is to evaluate how vital rates, such as growth or survival, change with temperatures. The main objectives of this study are: (1) to determine if factors affecting insect vital rates such as diet quality, developmental temperatures or acclimation also affect CT_{max} and (2) to determine if vital rates of different life stages (*i.e.*, insect larvae or adults) display different responses to temperature changes. If different life stages have particular thermal requirements, this may indicate different susceptibility to global warming. This study focuses on *Cephaloleia belti* (Coleoptera: Chrysomelidae), a tropical insect currently expanding its diet to an exotic host plant. We determined how high and low-quality diets (*i.e.,* native vs novel host), as well as exposure temperatures affect CT_{max} of adult beetles. We also estimated larval and adult survival when feeding on high and low quality host plants, when exposed to temperatures typical of the elevational distribution of this species, or when exposed to projected temperatures in 100 years. We did not detect an effect of diet quality or acclimation on CT_{max}. However, larvae and adults had different thermal requirements. CT_{max} is not affected by previous diet or acclimation as an adult. We propose that to understand processes involved in the adaptation and persistence of ectotherm populations in a warming world, studies must explore responses beyond CT_{max}, and focus on the response of vital rates to changing temperatures.

1. Introduction

Determining responses of organisms to changing temperatures is becoming a research priority as rapid global warming threatens populations and ecosystems around the world (Andrew et al., 2013; Gaston et al., 2009). To understand how changing temperatures affect organisms, it would be ideal to estimate how vital rates such as growth and survival are affected by novel thermal conditions (Gaston et al., 2009). In practice, measuring growth or survival is rarely implemented, as experiments would require estimating vital rates for multiple taxa and populations at different elevations, latitudes, and ecosystems (Garcia-Robledo and Horvitz, 2011).

An alternative approach for assessing thermal tolerance in ectotherms is to perform rapid laboratory assays that estimate the limits of an organism's thermal niche (Huey et al., 1992, 1989). A parameter used to determine upper thermal limits is the critical thermal maximum (CT_{max}), the highest temperature that organisms can tolerate before losing motor control (Folk et al., 2007; Lighton and Turner, 2004; Lutterschmidt and Hutchison, 1997; Terblanche et al., 2007). The critical thermal maximum is a parameter that is frequently used to compare relative tolerances to high temperatures among species and populations (Rezende et al., 2011). However, there is limited information on how factors affecting organisms' vital rates, such as diet quality or acclimation, affect intraspecific variation of CT_{max} (Vorhees and Bradley, 2012).

In this study, we were interested in determining how CT_{max} is affected by conditions known to reduce insect performance. In particular, we aimed to determine how diets of contrasting quality and

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temperature affect insect growth and survival, and if a relationship exists between vital rates and CT_{max} estimates.

We selected *Cephaloleia belti* (Coleoptera; Chrysomelidae) as our focal species. At our study site (La Selva Biological Station - Barva elevational gradient in Costa Rica, Central America), populations of *C. belti* have a wide elevational distribution: between 50 m and 2100 m above sea level (García-Robledo et al., 2016). The mean annual temperatures of the life zones inhabited by *Cephaloleia belti* range from 15 °C to 25 °C (Clark et al., 2015). The main host plant of *C. belti* is the native plant *Heliconia latispatha* (Zingiberales: Heliconiaceae). *Cephaloleia belti* is currently expanding its diet to *Musa velutina* (Zingiberales; Musaceae), an exotic host plant introduced to Costa Rica from India (Garcia-Robledo and Horvitz, 2012b).

Using *C. belti* as our focal organism, the main objectives of this study are: (1) to determine if factors affecting insect vital rates such as diet quality, developmental temperatures or acclimation also affect CT_{max} and (2) to determine if vital rates of different life stages (*i.e.*, insect larvae or adults) display different responses to temperature changes. If different life stages have particular thermal requirements, this may indicate different susceptibility to global warming.

In a first experiment, we determined the simultaneous effects of diet quality (high-quality native *vs.* low-quality novel host plants) and temperature on *C. belti* larval growth and insect survival. In a second experiment, we assessed if previous diet and acclimation affect the critical thermal maximum (CT_{max}) of *C. belti*.

Because tolerance to high temperatures involves a metabolic cost, we predicted that the CT_{max} would be reduced in insects experiencing challenging conditions such as diets that reduce their survival or extreme environmental temperatures (Lighton and Turner, 2004). One potential outcome is that the CT_{max} of insect herbivores will be reduced when feeding on lower-quality host plants or when exposed to extreme environmental temperatures that increase their mortality. Alternately, CT_{max} may be an intrinsic characteristic of insect species or populations. In this scenario, CT_{max} would remain constant independently from insect's physiological condition. If CT_{max} is not affected by an organisms' exposure to challenging environments, this parameter has the potential to estimate the intrinsic tolerance of invertebrates to high temperatures at local and global geographic scales.

2. Materials and methods

2.1. Study site and species

This study was conducted in 2017 at La Selva Biological station (hereafter La Selva), a tropical wet forest in the Caribbean lowlands of Costa Rica, Central America. La Selva receives approximately 4000 mm of rain per year, with a rainy season from May to December and a dryer season from January through March. The mean annual temperature of La Selva is 24.7 °C. Weather records from the past 50 years indicate an increase in temperature of 0.25 °C per decade (Clark, 2007; McClearn et al., 2016). La Selva is located at the base of the Barva Volcano, comprising the highest elevational gradient of continuous forest in Central America (McClearn et al., 2016). The La Selva - Barva transect ranges from 26 m to 2906 m above sea level and extends through four life zones within Braulio Carrillo National Park (Clark et al., 2015).

The neotropical beetle genus *Cephaloleia* (Chrysomelidae, Cassidinae) includes 214 known species distributed from Mexico to Central and South America (Staines, 2009; Staines and García-Robledo, 2014). This group is also known as the "rolled-leaf beetles" because adults feed and mate inside the scroll formed by the young leaves of plants in the order Zingiberales (García-Robledo et al., 2013). The temperature inside rolled leaves remains similar to surrounding air temperature (García-Robledo et al., 2016). However, leaf transpiration maintains the relative humidity inside the rolled leaf scrolls close to 100% (García-Robledo et al., 2016).

This study focuses on the La Selva population of the rolled-leaf beetle *Cephaloleia belti* (Chrysomelidae, Cassidinae). This beetle species is broadly distributed along the Barva transect from tropical rain forests at 26 m elevation up to tropical montane forests at 2100 m.a.s.l (Staines and García-Robledo, 2014). At La Selva, *C. belti* feeds mostly on the native host plant *Heliconia latispatha* (Heliconiaceae). *Cephaloleia belti* is currently expanding its diet to *Musa velutina* (Musaceae), an exotic plant species introduced to Costa Rica from southern India (Garcia-Robledo and Horvitz, 2011, 2012a, 2012b). Average development time from eclosion to pupation on the native host *H. latispatha* at ambient temperature is 39 days (Garcia-Robledo and Horvitz, 2011, 2012a, 2012b). Development time on the novel host *Musa velutina* is extended for an additional week (Garcia-Robledo and Horvitz, 2011, 2012a, 2012b).

2.2. Selection of diet and temperature treatments

To determine if diet quality affects *C. belti* larval growth, larval and adult survival, and CT_{max}, we selected two diet treatments: (1) the native host plant *Heliconia latispatha* (Heliconiaceae) and (2) the novel host plant *Musa velutina* (Musaceae). Previous studies established that the novel host *M. velutina* is a host plant of lower quality than *H. latispatha.* At ambient temperatures, *C. belti* larval growth is faster and adult survival is higher on *H. latispatha* than on *M. velutina* (Garcia-Robledo and Horvitz, 2011). Although differences in performance of *C. belti* on these two hosts are mediated by differences in the chemical composition of leaf tissue, we have no information on the secondary compounds present in leaves of these two species at our study site.

To determine the effects of temperature on *C. belti* larval growth, larval and adult survival, and CT_{max}, we selected three temperature treatments that represent the mean temperatures of each life zone at which *C. belti* is naturally present (tropical lower montane rain forest: 15 °C, tropical premontane rain forest: 20 °C and tropical wet forest: 25 °C). In addition, we included a fourth temperature treatment (30 °C). This treatment represents the environmental temperature increase that is expected under global warming projected over the next century in the lowland region of our study site (Clark, 2007; Clark et al., 2015; Colwell et al., 2008).

2.3. Larval growth on native and novel host plants at different temperatures

To determine the effects of diet and temperature on *C. belti* larval performance, we obtained 257 eggs from 20 gravid females. All females were collected in the lowlands from rolled leaves of *H. latispatha.* After eclosion, the length of each larva was measured using a camera attached to a dissection microscope (AmScope digital camera, Irvine, CA, USA).

Larvae were placed in individual containers and fed every 48 h with 4 cm^2 of leaf tissue from either the high-quality native host (*H. latispatha*) or the low-quality novel host (*M. velutina*). Larvae were reared in incubators at 15 °C, 20 °C, 25 °C, and 30 °C (*see* a description of the incubators built for this project in Supplement S1). We assessed the performance of larvae feeding on different diets and exposed to different temperatures by measuring larval growth after 15 d.

Differences in the growth of larvae exposed to different diets and temperatures were determined by performing a fully crossed ANOVA, with diet and temperature as the main factors. Differences among treatments were determined using *a-posteriori* Tukey HSD tests. All analyses were performed in Program R (R-Development-Core-Team, 2018).

2.4. Survival on native and novel host plants at different temperatures

To determine the effects of diet and temperature on larval survival, we recorded mortality of each larva included in the larval growth experiment. Larval mortality was recorded every 24 h for 15 d (*s*ample size in Table 2).