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Differences in winter cold hardiness reflect the geographic range disjunction of *Neophasia menapia* and *Neophasia terlooii* (Lepidoptera: Pieridae)

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ABSTRACT

Predicting how rapid climate change will affect terrestrial biota depends on a thorough understanding of an organism's biology and evolutionary history. Organisms at their range boundaries are particularly sensitive to climate change. As predominantly terrestrial poikilotherms, insects are often geographically limited by extremes in ambient temperatures. We compared the cold hardiness strategies of two geographically widespread butterflies, the pine white, *Neophasia menapia*, and the Mexican pine white, *N. terlooii* (Lepidoptera: Pieridae), at the near-contact zone of their range boundaries. Eggs are laid on pine needles and are exposed to harsh winter conditions. Eggs were collected from wild-caught butterflies, and we determined the supercooling point (SCP) and lower lethal temperature (LLT_{50}) of overwintering eggs. The SCP of *Neophasia menapia* eggs (−29.0 ± 0.6 °C) was significantly lower than that of *N. terlooii* eggs (−21.8 ± 0.7 °C). Both species were freeze-intolerant and capable of surviving down to their respective SCPs (LLT_{50} of *N. menapia* between −30 and −31 °C, *N. terlooii* between −20 and −21 °C). Cold exposure time did not affect the survival of *N. menapia*, but *N. terlooii* experienced somewhat greater mortality at sub-freezing temperatures during longer exposures. Our results, coupled with an analysis of microclimate data, indicate that colder winters in northern Arizona may contribute to the northern range limit for *N. terlooii*. Furthermore, careful analysis of historical weather data indicates that mortality from freezing is unlikely in southern Arizona but possible in northern Arizona. Movements of *Neophasia* range boundaries could be monitored as potential biological responses to climate change.

1. Introduction

Ambient environmental temperatures greatly affect the distribution of organisms. As small-bodied poikilotherms, insects are particularly affected by temperature (Sinclair et al., 2003), and low temperatures have a strong influence on insect distributions (Shi et al., 2012). Range boundaries have been shown to move north and upward in elevation in response to warming climate (Parmesan et al., 1999; Konvicka et al., 2003; Scriber, 2011). As such, movements of insect range boundaries can be useful indicators of biological responses to climate change.

The western North American arid regions are a natural laboratory for studying the influence of historical climate changes on the diversification of terrestrial biota (Smith and Farrell, 2005). Range expansions and contractions are a fairly common occurrence in the sky island region (Felger and Wilson, 1994), which encompasses the mountains of north-central Mexico and southeastern Arizona. Within this region, we focused on two butterflies: the pine white butterfly, *Neophasia menapia* (C. Felder and R. Felder, 1859), and the Mexican pine white butterfly, *N. terlooii* Behr, 1869. *Neophasia menapia* ranges from southwestern British Columbia to Guadalupe Mountains National Park in southwest Texas (Lotts and Naberhaus 2016; Scott 1986). *Neophasia terlooii* ranges from the sky islands of southeastern Arizona to the Sierra Madre of central Mexico (Bailowitz and Brock, 1990) (Fig. 1). The ranges of *N. menapia* and *N. terlooii* meet in central Arizona, with the former in the White Mountains and the latter in the sky islands, but there is no geographic overlap.

Mountain habitats for *Neophasia* spp. are susceptible to subfreezing conditions, with temperatures as low as −35 °C (National Centers for Environmental Information, 2016). In Arizona, *N. menapia* is univoltine and lays its eggs in July and August, while *N. terlooii* is bivoltine with the second generation laying its eggs in October. Eggs of both species are oviposited in linear clusters and overwinter on live pine needles.

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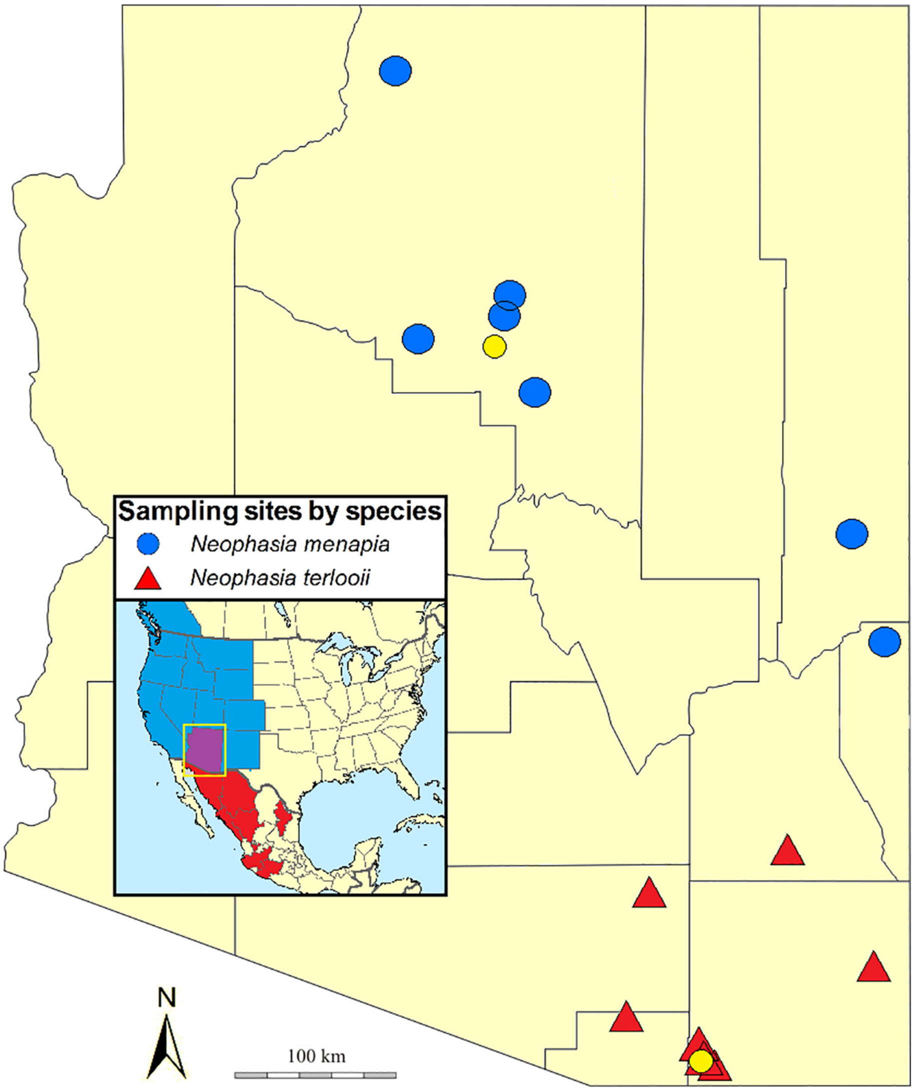

Fig. 1. Sampling sites for *Neophasia* in Arizona, with inset map showing geographic range of both species by state/province (there was one occurrence of *N. menapia* in extreme SW Texas): *Neophasia menapia* in blue and *N. terlooii* in red. Arizona is in purple to indicate the near-contact zone of both species. On the foreground Arizona map, blue circles indicate sampling sites for *Neophasia menapia*. Red triangles indicate sampling sites for *N. terlooii*. Yellow circles indicate where iButton temperature data were collected, with the northernmost circle representing two trees in Flagstaff and the southernmost circle representing one tree in Sawmill Canyon. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Pine forests are known to be sensitive to climate change (Allen and Breshears, 1998), but the long generation time of trees makes them poor indicators of rapid, ongoing responses to recent climate change. Thus, *Neophasia* spp., with their close association with pine trees, coupled with their abundance, ease of detection, and rapid annual life cycles, are prime candidates for indicators of biological responses to rapid climate change among insects in pine forests.

We investigated the cold hardiness strategy and limits of survival for both *Neophasia* spp. Among insects, three cold hardiness strategies are typically recognized (Lee, 2010): 1) Freeze-tolerant species can survive internal ice formation, 2) Freeze-intolerant species must remain in a supercooled state and are killed by any internal ice, and 3) Chill-susceptible species succumb to cold injury well above the supercooling point (SCP; i.e., the temperature at which internal ice nucleation occurs). A majority of insects are incapable of freezing and depress their supercooling point by removing potential ice nucleating agents, synthesizing cryoprotectants and, in some cases, producing antifreeze proteins (Lee 2010). By comparing the SCP to the lower lethal temperature (e.g., LLT_{50}, the temperature at which 50% of individuals die), the cold hardiness strategy can be empirically determined (Sinclair et al., 2015). Intensity and duration of exposure interact to determine survival – prolonged exposure to temperatures above the SCP can result in mortality, even in freeze-intolerant insects with low SCPs (Delisle et al., 2013).

Cold hardiness can limit the geographic ranges of insects (Shi et al., 2012). In our system, two congeners have disjunct distributions near a major ecotone, despite the fact that the host plants for both species overlap at this ecotone (Southwest Environmental Information Network, 2016). Our goal was to investigate differences in cold hardiness and compare them to differences in temperature profiles at the ecotone. We propose that a lower level of cold hardiness in *N. terlooii* may contribute to its northern range limit. We then use the results of this study to discuss mechanisms of range disjunction and implications for using *Neophasia* as bioindicators of the effects of climate change on forest-dwelling insects. *Neophasia* spp. are ubiquitous members of pine forest communities in western North America, at times reaching outbreak numbers (Ciesla, 1974), and our study is the first to empirically investigate their thermal biology.

2. Materials and methods

2.1. Collecting adult specimens

Sampling took place at similar elevations and latitudes in Arizona, where the ranges of both species are in closest proximity. To account for cold hardiness variations within each species, butterflies were collected from seven sites along their range boundaries (Fig. 1). Butterflies used for egg production were collected during the peak flight seasons of adults in 2013–2015: July/August for *N. menapia* and October for *N. terlooii*. Field-collected butterflies were stored individually in glassine