Freeze tolerance: comparing two geographically isolated populations of the cinnabar moth

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Abstract
Freezing to death in winter is a danger faced by ectothermic organisms throughout temperate climates. One adaptation of lepidopteran insects for overwinter survival is the sequestration of antifreeze solutes in the hemolymph prior to pupal diapause. This acts to depress freezing points during the long and immobile pupal stage during winter. We measured freezing (“supercooling”) points of two geographically isolated populations (valley versus mountain) of the cinnabar moth, Tyria jacobaeae (Lepidoptera: Erebidae) to test if mountain populations have evolved lower freezing points than valley populations. The hypothesized differences between the two populations are due to rapid (<30 generations) adaptive evolution following the insect’s recent colonization of the harsher mountain climate.

Fig 1 Tyria jacobaeae in pupal form

Introduction
Biological control insects provide opportunities to test for adaptations to new climates. The present study involves tansy ragwort (Asteraceae: Jacobaea vulgaris), an invasive and poisonous weed accidentally introduced to western Oregon (1). To combat its spread, the cinnabar moth was released as a biological control agent first in lowland sites of Oregon beginning in 1960 and then mountain clear-cut logging sites throughout the 1980s. The source populations used for mountain redistributions of the moth were sites of Oregon beginning in 1960 and then mountain clear-cut logging sites throughout the 1980s. The source populations used for mountain redistributions of the cinnabar moth, Tyria jacobaeae, were released beginning in 1960 and the insect continues to thrive despite greatly shortened summers, and colder, longer winters where temperatures routinely drop below -15°C. Therefore this system allows us to investigate adaptive changes in cold hardiness between the ancestral (valley) and derived (mountain) population within a known timeline.

Methods
We reared eggs and larvae collected from high elevation sites in the Oregon Cascades (“mountain,” ~1400 meters) and low elevation sites in the Willamette Valley, Oregon (“valley”, ~50 meters). We separated pupae into three prewinter treatment groups: 0, 1, and 2 (representing no prewinter, mountain prewinter condition, and valley prewinter condition, respectively, Fig 2).

Upon pupation, treatment groups were subjected to 22°C day and 7°C night temperatures and 15h light:9h dark photoperiod in controlled environmental chambers for either 7 days (Treatment Group 0), 67 days (Treatment Group 1), or 127 (Treatment Group 2) consecutive days. All treatment groups were then held for 30 days in constant 5°C and 0L:24D to simulate one month of winter.

Results
Prewinter acclimation time had no effect on supercooling points for either population. (F = 1.96, d.f. = 118, P = 0.145; Fig 4).

Fig 4 Summary of supercooling points.

When acclimation groups were pooled, valley origin insects had significantly lower supercooling points than mountain origin insects (t = 2.3952, df = 119.99, P = 0.01816). Valley pupae froze at -23.19°C (mean, ±3.40SD, n = 57), while mountain pupae froze at -21.62°C (±3.85 SD, n = 65).

The populations appear not to differ in pupal length (t = -1.3198, df = 119.58, P = 0.1894) or pupal width (t = 0.0512, df = 119.99, P = 0.9593), however our data suggest pupal body size is correlated with supercooling points. Longer pupae had lower supercooling points (Fig 6A), which decreased by 1.329°C for every one millimeter increase in its length (F = -3.084, P = 0.00253). Pupal width had a similar effect on supercooling point (Fig 6B), however millimeter increase correlated with a 1.8898°C decrease in supercooling point (F = 2.775, P = 0.00641). A direct causal relationship between pupal size and supercooling points is questionable however, given the large residuals and correspondingly low coefficients of determination associated with the linear regression models.

Discussion
No difference among acclimation groups:

- Freeze tolerance might not require extended periods of preparation or...
- Follow-up tests that eliminate the 30-day “cool-down” period (see ‘Methods’) may reveal differences among prewinter acclimation intervals within, or between, populations when snap-freezes occur.

Why are valley populations more freeze tolerant? Is it not colder in the mountains?

- Yes, but there is also more snow, which may act as a thermal buffer to sub-zero temperatures. Thus, effective (pupal body) temperature may in fact be colder in the valley. Empirical data collection is planned for testing this.
- Mountain populations have largely shifted from the target host ragwort to a novel host arrowleaf groundsel (Senecio triangularis). This inferior host may affect physiological mechanisms of freeze tolerance.

Fig 5 Adult T. jacobaeae after emerging sometime in Spring

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References