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Brief Communication

Canalization of freeze tolerance in an alpine grasshopper ☆

Timothy C. Hawes *

Khon Kaen University, 123 Mitraphap Highway, Khon Kaen 40002, Thailand
Department of Zoology, University of Otago, Dunedin, New Zealand

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ABSTRACT

In the Rock and Pillar Range, New Zealand, the alpine grasshopper, *Sigaus australis* Hutton, survives equilibrium freezing (EF) all-year round. A comparison of freeze tolerance (FT) in grasshoppers over four austral seasons for a 1 year period finds that: (a) the majority (>70%) of the sample population of grasshoppers survive single freeze-stress throughout the year; (b) exposure to increased freeze stress (multiple freeze-stress events) does not lead to a loss of freeze tolerance; and (c) responses to increased freeze stress reveal seasonal tuning of the FT adaptation to environmental temperatures. The Rock and Pillar sample population provides a clear example of the canalization of the FT adaptation. Seasonal variability in the extent of tolerance of multiple freezing events indicates that physiology is modulated to environmental temperatures by phenotypic plasticity – i.e. the FT adaptation is permanent and adjustable.

1. Introduction

The freeze tolerance (FT) adaptation may be said to be completely canalized in an insect when the majority of a sample population of a specific life history stage are capable of consistently surviving the equilibrium freezing (EF) of body fluids across a spectrum of thermal contexts. In other words, the cryotype does not change: it is fixed. This study describes an instance of such complete canalization in the alpine grasshopper, *Sigaus australis* Hutton, from the Rock and Pillar Range, New Zealand.

Complete canalization is often side-stepped by FT species. For example, many respond to seasonal thermal heterogeneity via the employment of different 'winter' and 'summer' strategic responses to temperature. The FT adaptation may be abandoned or not expressed out of preference for strategic alternatives like phenotypic plasticity, seasonal life history partitioning, and bet hedging. Additionally, some insects may be said to exist in a pre-canalized state in which the adaptation has been incompletely acquired [5]. Just as pre-canalization may be expressed in terms of the extent to which EF is 'incompletely' tolerated [5], so may the extent of derivation in a canalized organism be expressed in terms of the extent to which EF is 'completely' tolerated. In other words, how much EF can a FT insect withstand? The methodological correlate to this question – the repetitive or consecutive freezing of an FT insect – is a method pioneered by Bale et al. [1] for examining cryo-stress in FT-adapted insects.

This study set out to identify if, and at what level, the New Zealand alpine grasshopper, *S. australis*, exhibits variability in its tolerance of the freezing process itself: across seasons and through single and multiple freeze exposures. It was hypothesized that because *S. australis* may be exposed to low temperatures and freezing events throughout the year, it has acquired a fixed cryotype in which freeze tolerance is completely canalized.

Adult female grasshoppers were collected from the Rock and Pillar Range, South Island, New Zealand. *S. australis* was identified with reference to Bigelow [2]. Samples were collected over four austral seasons through 2008–9: Winter = May 2008; Spring = September 2008; Summer = December 2008; Autumn = March 2009. Grasshoppers were returned to the laboratory and kept in plastic boxes at 5 °C with moistened cotton wool and a loose bedding of grasses, leaves, and soil taken simultaneously from the collection site.

Grasshoppers were exposed to three freeze cycles in which replicates of 8 individuals were frozen to equilibrium once (F1), twice (F2) or three (F3) times. Equilibrium freezing was defined as the freezing of all available body water. Experimentally, this was measured as the completion of the exotherm as described by Hawes and Wharton [5] and Hawes [6]. This was done for each of the four seasonal samples with comparisons between seasons and freeze treatments (F1–F3). Temperature of equilibrium

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* Address: Khon Kaen University, 123 Mitraphap Highway, Khon Kaen 40002, Thailand.

E-mail address: timothyhawes@hotmail.com

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freezing (T_{ef}) and survival of freezing were measured using a cooling rate of 0.5 min^{-1}. T_{ef} was measured individually for each grasshopper using the method described by Hawes [6] to determine core body temperature at the point at which the freezing of body fluids had reached equilibrium, with grasshoppers removed 0.5 °C below T_{ef} [5]. After each freeze cycle grasshoppers were given 48 h to recover and then survival of EF was assessed. Moribund individuals were readily identifiable as animals unable to right themselves, or stuck on their back, or incapable of movement beyond the twitching of their legs. Survivors were responsive to gentle prodding stimuli from a paintbrush, could turn themselves over, and, more often than not, attempted to jump out of their containers. Grasshoppers were maintained individually in transparent plastic chambers (diameter 65 mm, height 82 mm) with a perforated lid, a few leaves and two to three balls of damp cotton wool to prevent dehydration. The freezing protocol was repeated for F1–F3 for each seasonal sample set. Thus, F1 individuals that were scored as alive after 48 h, were transferred to F2 treatment; and F2 individuals that were scored as alive after 48 h, were transferred to the F3 treatment. N for survival assessments is counted as the total number of grasshoppers frozen for each treatment minus a subset of 8 grasshoppers that were removed after the F1 and F2 treatments and frozen for subsequent laboratory analysis (TCH, unpublished data). (An additional 8 grasshoppers were removed after the F3 treatment but this did not affect sample numbers as the experiment was completed at this stage). N for each treatment was therefore: F1 = 40; F2 = 32; F3 = 24.

Data for both T_{ef} and survival were not normally distributed and could not be transformed. The Kolmogorov–Smirnov test was used to compare empirical distributions across seasons and treatments for T_{ef}. Proportions of sample populations that survived freezing were compared between seasons using the Chi-squared test (categories of comparison = 'live' vs. 'dead'). Comparisons were made between like treatments only (e.g. F1 vs. F1) so as not to violate assumptions of independence, where survival in later freeze

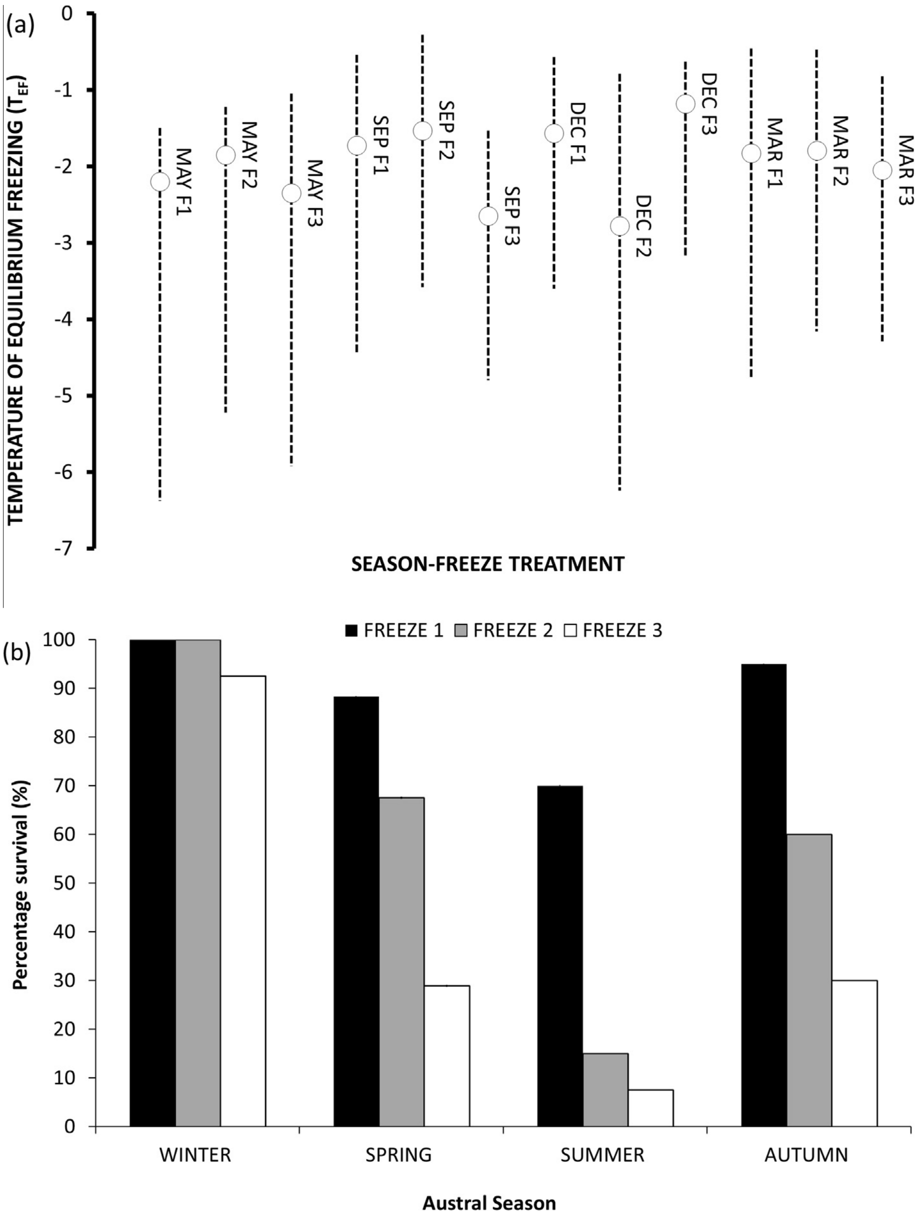

Fig. 1. (a) Temperature of equilibrium freezing (T_{ef}) of the grasshopper, *S. australis*, with medians (white circles) and first and third interquartiles (dashed line); (b) percentage survival (%) of equilibrium freezing by the grasshopper, *S. australis*, in relation to season and freeze treatment.

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