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Cold-acclimation increases the predatory efficiency of the aphidophagous coccinellid *Adalia bipunctata*

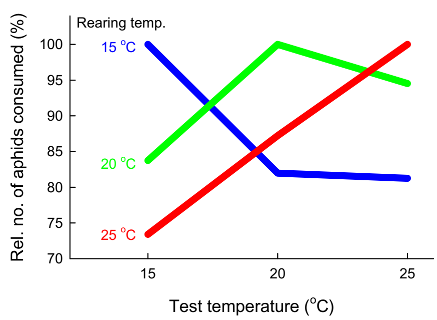
Christian Hougaard Sørensen [a], Søren Toft [a,*], Torsten Nygaard Kristensen [a,b]

[a] *Department of Bioscience, Aarhus University, Ny Munkegade 116, DK-8000 Aarhus C, Denmark*
[b] *Department of Molecular Biology and Genetics, Aarhus University, Blichers Allé 20, DK-8830 Tjele, Denmark*

HIGHLIGHTS

- ▶ Cold acclimation may increase the biocontrol efficiency of a ladybird.
- ▶ Acclimated beetles had the highest biocontrol efficiency at all temperatures.
- ▶ Cold acclimation increased body-size but reduced pupal survival and heat resistance.
- ▶ Acclimation should be considered in augmentative release programs.

GRAPHICAL ABSTRACT



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ABSTRACT

Ladybirds are used in integrated pest management and augmentative biological control programs all over the world. Typically, commercial rearing of the commonly used ladybird, *Adalia bipunctata*, takes place at a constant temperature (25 °C) which maximizes reproductive output and survival in the laboratory. However, insects are known to acclimate via physiological adjustments to their thermal environment and performance is often higher at temperatures to which they are acclimated. Thus rearing *A. bipunctata* at 25 °C may not be optimal if they are to effectively manage aphid pests under different thermal regimes. Here, we report on the effects of rearing temperature (15, 20 and 25 °C) of *A. bipunctata* on aphid predation at similar test temperatures and under cold semi-natural conditions. Furthermore we assessed the upper thermal critical limit of ladybirds from the three rearing temperatures using a heat knock down assay as well as the effects of rearing temperature on pupal survival and adult mass. We demonstrate that ladybirds acclimated to a certain temperature consume more aphids at that temperature than ladybirds acclimated to other temperatures. Acclimating ladybirds to cold temperatures also increased their body-size but reduced pupal survival and heat resistance, suggesting costs associated with acclimation. Our findings have implications for the application of ladybirds as bio-control agents in different thermal environments. The results can be used to improve the efficiency of pest management in biological control programs.

1. Introduction

Predaceous ladybirds (family Coccinellidae) have received attention from ecologists, because of their use in biological control as predators of agricultural pests e.g. aphids, diaspids, coccids, aleyrodids and mites (Obrycki and Kring, 1998; Omkar and Pervez, 2005). They have been used as a component of integrated pest management and in augmentative control programs since the early 20th century (Hodek, 1970). Today, ladybirds are commercially produced and sold as bio-control agents, in particular against aphids which damage for billions of dollars of crops annually worldwide (Oerke, 1994). *Adalia bipunctata* (L.) is one of the best

* Corresponding author. Fax: +45 87154326.
E-mail address: soeren.toft@biology.au.dk (S. Toft).

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studied ladybirds due to its potential use against aphid pests and because it is one of the most common aphidophagous predators in arboreal habitats of Europe and Central Asia (Hodek and Hoňek, 1996).

Because *A. bipunctata* is used in augmentation biocontrol programs, it is of interest to optimize its efficiency against aphid pests. Population growth parameters reported for *A. bipunctata* indicate that the species may be useful both in greenhouse systems (e.g. against *Myzus persicae* Sulzer) and in a number of outdoor crops (e.g. against *M. persicae* and *Acyrthosiphon pisum* Harris) (Jalali et al., 2009). Therefore, individuals released for bio-control measures are exposed to a wide range of temperatures. Temperature is an important factor influencing developmental rate, survival, adult size and feeding activity of many insects (Wratten, 1973; Schüder et al., 2004; Jalali et al., 2010). The predation rates of larval and adult stages of *A. bipunctata* on aphids increase with temperature in the range of approximately 10–30 °C (Gotoh et al., 2004). Typically, commercial rearing of *A. bipunctata* use only a single constant temperature (25 °C), which is optimal for reproduction, survival and development (BioBest, Belgium, Carl De Coninck – personal communication; Schüder et al., 2004). The thermal rearing regime may affect their ability to effectively manage aphid pests when employed at temperatures well below (or above) temperatures that are optimal in the laboratory.

One method that may help to improve the efficiency of predators in biological control programs is thermal acclimation (e.g. Chidawanyika and Terblanche, 2011; Hart, 2002). Acclimation is often defined as a phenotypic alteration in physiology that occurs in response to the environmental conditions experienced by an animal and is often thought to enhance performance, thereby improving fitness (Angilletta, 2009). This view has been termed the *beneficial acclimation hypothesis* which predicts that "... acclimation to a particular environment gives an organism a performance advantage in that environment over another organism that has not had the opportunity to acclimate to that particular environment" (Leroi et al., 1994). Although the beneficial acclimation hypothesis is controversial and has been rejected as a general rule (Gibbs et al., 1998; Krebs and Loeschcke, 1994; Woods and Harrison, 2001), many studies on acclimation responses, especially those dealing with temperature, have supported the hypothesis (Chidawanyika and Terblanche, 2011; Nunney and Cheung, 1997; Thomson et al., 2001). For instance Chidawanyika and Terblanche (2011) showed that thermal acclimation can give mass-reared codling moths, *Cydia pomonella*, a significant performance advantage when released at temperatures similar to their developmental temperature. However, it may be costly to acclimate to a certain environment if it leads to trade-offs in performance under different thermal conditions (Chevin et al., 2010; Kristensen et al., 2008; Loeschcke and Hoffmann, 2007). Evidence for trade-offs were found in a study by Kristensen et al. (2008), testing for effects of larval and adult cold-acclimation on field released *Drosophila melanogaster*. At low release temperatures, flies acclimated at 15 °C were recaptured at baits almost 100 times more often than flies acclimated at 25 °C, indicating strong benefits of cold-acclimation to cope with cold conditions in the field. However, this advantage came at a huge cost at higher temperatures, where flies acclimated at 25 °C were up to 36 times more likely to find food than flies acclimated at 15 °C. Thus, costs and benefits of acclimation are contingent on the conditions the animal encounters subsequently.

The aim of this study was to test the effects of acclimation to three different developmental temperatures in a common aphidophagous predator, *A. bipunctata.* To fulfill this objective, we examined the ability of ladybirds, previously acclimated to developmental temperatures of 15, 20 or 25 °C, to consume aphids at four different temperature regimes (constant 15, 20 or 25 °C and one at fluctuating temperatures with a mean of 8.5 °C). We concentrate on responses to low acclimation temperatures since this is most representative for outdoor thermal conditions that are prevailing in Central and Northern Europe. The ability of *A. bipunctata* to control aphids was tested using a microcosm design which partly simulates the complex habitat structure of a cereal field. Furthermore, we tested acclimation effects on heat resistance in order to determine whether or not acclimation to low temperatures entails costs in terms of reduced ability to withstand high temperatures. Developmental effects of the temperature treatments were also investigated by scoring pupal survival and the mass of the adult ladybirds.

We hypothesized that (1) developing at a particular temperature enhances the predatory performance of ladybirds in terms of an increased feeding rate on aphids at that temperature (according to the beneficial acclimation hypothesis); (2) that acclimation to low temperatures imposes a cost to the ladybirds in the form of reduced heat resistance; (3) that there is an association between developmental temperature and body-size with beetles maturing to larger sizes at lower developmental temperatures, as found in many other studies (e.g. Alpatov, 1930; Azevedo et al., 2002) and (4) that pupal survival is highest at 25 °C since this temperature is considered optimal for the species (Schüder et al., 2004). We confirmed all four hypotheses and discuss the implications of our results for the application of ladybirds as bio-control agents in different thermal environments and for the efficiency of biological control systems in general.

2. Materials and methods

2.1. Predator culture

A. bipunctata larvae were purchased from Biobest NV (www.biobest.be). The colony in our laboratory was infused with new individuals from the same commercial source three times during establishment in our laboratory. In our experiments we only used the melanic form, *quadrimaculata* (black with four red spots) which comprised approximately 80% of the emerging adults.

At Biobest, all lifestages were continuously reared at 25 °C and fed pea aphids, *A. pisum*. In our laboratory, the ladybirds were reared for two generations on an *ad libitum* supply of grain aphids, *Sitobion avenae* (Fabricius), prior to experiments. During that time the stock colony of the predator was maintained in a growth chamber at 25 ± 0.5 °C, and a 17:7 L:D photoperiod. Individual larvae were isolated in plastic tubes (height 8.1 cm; diameter 3.4 cm) containing a piece of dry filter paper. The larvae were fed aphids offered *ad libitum* on wheat leaves three times a week.

2.2. Prey culture

For all experiments, *S. avenae* was obtained from a laboratory culture, reared at room temperature (ca. 22 °C) and a 16:8 L:D photoperiod on wheat seedlings of mixed cultivars. The culture was purchased at EWH BioProduction ApS (www.bioproduction.dk) where they also were reared on wheat seedlings. The grain aphid is known to be a suitable prey species for *A. bipunctata* that supplies the predator with all essential nutrients (Schüder et al., 2004).

2.3. Microcosm study

To measure predator performance two separate microcosm experiments were run; one at three constant temperatures in the laboratory (15, 20 and 25 °C), and one at natural fluctuating temperatures in an outdoor environment. The two microcosm experiments were run at different dates and varied in some details of their experimental design. The microcosm set-up was roughly