Seasonal cold adaptation dynamics of some carabid beetle species: *Carabus granulatus*, *Pterostichus oblongopunctatus* and *Platynus assimilis*

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Abstract. Cold-hardiness can be measured by supercooling points – the temperature at which spontaneous freezing occurs. Seasonal changes in supercooling point were assessed in field-collected predacious carabid beetle species: *Carabus granulatus* L., *Pterostichus oblongopunctatus* L. and *Platynus assimilis* Payk. (Coleoptera: Carabidae). Supercooling ability of these beetles changed seasonally. The tested carabid beetles proved to belong to freeze-avoiding cryotype.

Key words: Coleoptera, Carabidae, cold-hardiness, supercooling.

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Introduction

Seasonal adaptation of insects depends on many abiotic factors, with temperature being the most critical factor aside of humidity and light (Danks, 2006, 2007). Insects survive low temperatures either by keeping their body fluids liquid below their ordinary freezing point (freeze avoidance), or by surviving the formation of ice in their tissues (freeze tolerance) (Bale & Hayward, 2010; Lee, 2010). For species inhabiting in temperate and colder climates, the ability to supercool is undoubtedly the most important component of the overwintering strategy. At temperatures below 0 °C, most insect species remain unfrozen because they supercool. Cold-hardiness can be measured by indices such as supercooling points (SCP), the temperature at which spontaneous freezing occurs (Danks, 2004).

Freeze-avoiding insects keep their body

fluids liquid by removing ice nucleators that initiate ice formation, synthesizing antifreeze proteins to reduce the nucleation potential of seed crystals, and accumulating sugars and polyols, such as glycerol or trehalose, which also lower the crystallization temperature (defined as its supercooling point) and stabilize membranes at low temperatures (Vernon & Vannier, 2002; Lee, 2010).

Freezing-tolerant insects survive ice formation in body tissues. Ice formation usually is confined to extracellular fluids, thus avoiding damage to intracellular components. Protective mechanisms for freezing resistance include elevated solute levels, presence of nucleating agents, and accumulation of cryoprotectants in body fluids. A number of freeze-tolerant insects have supercooling points in the range of –8 °C to –10 °C, whereas some freeze-avoiding species supercool extensively, even to –60 °C or below. Insects that supercool are freezing-

Figure 1. The generalized diagram of insect response to subzero temperatures. Insect body temperatures (heavy line) in relation to the melting point, the supercooling point, and the nucleation of ice in body fluids. The bars on the right convey general ranges of insect response to low temperatures, the top of the bar for the range of freeze tolerance and the bottom of the bar for freeze avoidance correspond to the supercooling point value illustrated in the center of the figure (Lee, 1989; Ellsbury *et al.*, 1998).

Joonis 1. Putukas toimivate temperatuurist põhjustatud muutuste näitlik diagramm (Lee, 1989; Ellsbury *et al.*, 1998). Tugev must joon näitab putuka kehatemperatuuri (body temperature) muutusi temperatuuri langedes; viirutatud ala vastab jää (ice) tekkele ja olemasolule putuka organismis. Kehavedeliku sulamispunkt (melting point of body fluids) on temperatuur, mille juures sulab viimane kehas olev jääkristall. Enne allajahtumispunkti (supercooling point) ehk tegelikku külmumistemperatuuri on kehavedelikud (supercooled fluids) 0 °C madalamas temperatuuris jahtunud olekus. AJP (allajahtumispunkti) juures eraldub kudedes spontaanselt külmumisel soojust (heat of crystallization). Putukate seisundid temperatuuri langemisel: normaalne aktiivsus (normal activity), külmašokk (cold shock), külmumise vältimine ehk külmatundlikkus (allajahtumine) (freeze avoidance (supercooled)), külmumise talumine (freeze tolerance). Külmašokk on jahtumine ilma külmumata, mõne külmatundliku putuka jaoks on pikaajaline madal temperatuur letaalne. Toimuvad muutused on tihedalt seotud aja- (time) ja temperatuurifaktoriga (temperature).

avoiding since they avoid freezing damage to body tissues only at a temperature above the supercooling point. Below the supercooling point, death results from ice formation in body tissues (Danks, 2004). These relationships are summarized in Figure 1 adapted from Lee (1989) and Ellsbury *et al.* (1998).

Cold-hardiness is equally important to pests and beneficial predators, includ-

ing carabid beetles known of their sensitive reaction to environmental changes (Koivula, 2011). Carabid beetles (Coleoptera: Carabidae) are species rich and abundant in arable and forest habitats (Kromp, 1999). Carabid beetles are natural enemies attacking also both immature and adult pine weevils (Salisbury & Leather, 1998; Dillon & Griffin, 2008). Adult carabid beetles exhibit scarcely any activity in winter. Carabid

species that inhabit cultivated fields spend the winter in soil or migrate into the field boundaries, whereas some species migrate into the forest (Thiele, 1977). The microenvironment of overwintering sites is at least as important in allowing winter survival as are physiological adaptations (Danks, 2004).

The aim of the present research was to study seasonal cold adaptation dynamics of carabid beetles *Carabus granulatus* L., *Pterostichus oblongopunctatus* L. and *Platynus assimilis* Payk. These species are fairly common, all univoltine spring breeders (Luff, 1993) and represent three Carabidae genera. As they overwinter in older and partially decayed tree stumps they can be used as model species for Carabids of similar biology associated with both agricultural and forest ecosystems.

Materials and methods

Insects

Laboratory experiments were conducted to test three carabid species: *Carabus granulatus* (Linnaeus 1758), *Pterostichus oblongopunctatus* (Linnaeus 1758) and *Platynus assimilis* (Paykull 1790). Adult insects were collected from their hibernating sites – tree stumps at wintertime (January) and from the field by using dry pitfall traps in spring (May) and autumn (September). Beetles were collected from the Tartu County, Estonia (ca. 58°26' N, 27°7' E) in 2012. Samplings were performed early in the morning in cool weather (temperatures between 0 °C to 10 °C) and beetles were placed in the refrigerator at 4 °C immediately after the arrival to avoid major temperature fluctuations. To obtain field-collected beetles with empty guts, they were held for 24 h prior to the testing without food in plastic boxes filled with moisturized moss (for retaining moisture and providing hiding areas).

Experimental design

Together 216 beetles were recorded (9 variances (3 months × 3 species) × 24 insect replicates), the sex of insects were not deter-

mined. For measuring supercooling points (SCPs), the beetles were positioned so that its integument (thoracic tergite) was in contact with the copper-constantan thermocouples-thermometer (RS-232 Data logger thermometer; TES Electrical Electronic, Taiwan), placed in glass vial closed by cap, and then transferred to circulator bath (Ministat 230w-2, Huber, Germany, -33 °C to +200 °C). SCP was determined using a 0.5 °C min⁻¹ cooling rate. The SCP was taken as the thermocouple recorded the lowest point before the emission of the latent heat of crystallization (when body fluids freeze). From each species five individuals were taken for determination of cold-hardiness strategies (freeze-tolerant or freeze-avoidance) by supercooling. When the temperature limit to supercooling is reached, heat of crystallisation appears, species who will survive recooling to the SCP have freeze-tolerant overwintering strategy, whereas those who will not survive are classified as freeze-avoiding species (Lee, 1989; Bale, 2002). The temperature curve was registered and saved by using data logger (Almemo 2890-9, Ahlborn Mess- und Regelungstechnik GmbH, Germany).

Statistical analysis

Supercooling points were tested for normality using the Kolmogorov-Smirnov Test. Statistical comparisons of the seasonal change of mean supercooling points of individuals were performed with repeated-measures one-way analysis of variance (ANOVA) followed by Fisher's LSD test, using STATISTICA 12 package. The collected data were analysed separately in each species. All means were considered significantly different at the $p \leq 0.05$ level.

Results and discussion

Mean supercooling points are shown in Figure 2. Seasonal mean SCPs examined in *C. granulatus* were determined as follows: -5.4 °C (in January), -5.2 °C (in May) and -6.3 °C (in September). The SCP in autumn

Figure 2. Supercooling seasonal dynamics of the carabid beetles *C. granulatus*, *P. oblongopunctatus* and *P. assimilis*. Columns with different letters are significantly different (ANOVA Fisher's LSD test, $p \leq 0.05$). The differences were calculated between one species. Box-and-whisker plots: boxes show standard errors, whiskers are standard deviation bars, small box in the centre is mean.

Joonis 2. Sõmerjooksiku (*C. granulatus*), metsa-süsijooksiku (*P. oblongopunctatus*) ja süsi-ketasjooksiku (*P. assimilis*) külmakindluse sesoonne dünaamika. Allajahtumispunkte (supercooling point °C) on võrreldud liigi piires. Statistiliselt usaldusväärsed erinevused ($p \leq 0.05$) on tähistatud erinevate tähtedega (ANOVA Fisher's LSD test).

differed significantly from that in January ($F_{2,69} = 14.17$, $p \leq 0.05$) and in May ($p \leq 0.05$), but there were no difference between winter and spring SCPs ($p = 0.43$). The SCPs of *P. oblongopunctatus* ranged from -6.3 °C (May) to -7.2 °C (September). Similarly to *C. granulatus*, supercooling of *P. oblongopunctatus* was lower in autumn compared to winter ($F_{2,69} = 25.49$, $p \leq 0.05$) and spring ($p \leq 0.05$), but also no differences between winter and spring supercoolings were found ($p = 0.44$). The SCPs of *C. granulatus* and *P. oblongopunctatus* reached their minimum during autumn, and then raised by mid-winter to values as high as in spring. This pattern, which matches the autumn-dynamic strategy characterized by Merivee (1978), could be an physiological adaptive response in association with behavioral patterns of these species in Northern temperate condition. According to the autumn-

dynamic strategy the greatest changes in cold-tolerance take place in autumn – the ability to supercool attains its winter maximum as early as autumn, in September–October. In the course of winter (from November to April) it does not change noticeably. Supercooling capacity changes during the year and this is increasing when insect prepares for resting stage. Recorded SCPs were higher in January, compared to September. It is now known that many species complete diapause by mid-winter, but development is still suppressed by continuing low temperature. In such species, and those without a winter diapause, polyol concentrations and low SCP are usually maintained until spring, indicating that rising temperature triggers the reversible synthesis or degradation of the carbohydrates and consequent loss of cold-hardiness (Leather *et al.*, 1993).

The SCPs were lowest in the beginning of the overwintering (in September) in all examined insects: in *C. granulatus* -6.3 ± 0.73 °C; in *P. oblongopunctatus* -7.2 ± 0.41 °C and in *P. assimilis* -9.3 ± 1.58 °C. In *P. assimilis*, beetles collected in May produced highest SCP (-6.8 °C), three months earlier in January the SCP was -8.7 °C. There were significant differences between all the seasonal SCPs of *P. assimilis* beetles ($F_{2,69} = 27.17$, $p \leq 0.05$). Earlier research by Kivimägi *et al.* (2009) has measured the January SCP at -5.5 °C on *P. assimilis*, which indicates that the SCP and thus the cold-hardiness is regulated by environmental conditions and depends on the physiological state of the insect (Marshall & Sinclair, 2012). For example carabid beetle *Pterostichus brevicornis* (Kirby 1837) was studied in Alaska, where it overwinters in decaying tree stumps and felled timber, although the habitat is at times partly covered with snow. These beetles may experience temperatures as low as -60 °C, with prolonged exposure to -60 °C over several weeks. Seasonally collected beetles showed SCPs from -6 °C in summer (when freezing was fatal) to -11 °C, when beetles became freeze-tolerant (Baust & Miller, 1970). The most striking feature of freezing intolerant insects is the seasonal increase in supercooling from summer through autumn to winter. Hiiesaar *et al.* (2012) found that supercooling ability of horseradish flea beetles *Phyllotreta armoraciae* (Koch 1803) is depending on their feeding status. In nature, most overwintering insects of the temperate or colder climates evacuate the digestive tract as a part of a multifactorial process that maximises supercooling in winter.

The tested carabid beetle species didn't survive the recooling until SCP, therefore they belong to freeze-avoiding insect group (Lee, 1989; Bale, 2002). The large majority of terrestrial arthropods that have been investigated are freeze-avoiding. Freeze-avoiding species tend to (1) choose thermally buffered microsites for overwintering and (2) lower the temperature at which

ice formation occurs (supercooling point) by producing antifreeze agents, minimizing internal nucleation sites, and avoiding contact with external ice (Sømme, 1999). Unseasonal cold in summer could cause some mortality in freeze avoiding insects, as there is not enough time for such insects to clear their guts and undergo the biochemical changes associated with the long-term acclimation for winter conditions (which is the process that may take days or weeks) (Sinclair *et al.*, 2003). All the studied insect species overwinter in protected place – in tree stumps. After moving to overwintering sites beetles stop feeding, enabling them to increase their supercooling capacity. The greatest risk of mortality from low temperatures comes from the combination of no snow and low temperatures. Snow is an important insulator against low temperatures and a cover of snow or vegetation also helps to protect overwintering individuals against lethal temperatures, therefore snow cover could have significant matter in insects overwintering (Danks, 2004; Bale & Hayward, 2010). According to the Estonian Meteorological and Hydrological Institute's (EMHI) measurements, the mean air temperature in insects overwintering time in January 2012 was -5.8 °C and snow cover 23 cm. Consequently, overwintering mortality due to low temperature may be rare, for example, only in extremely severe snow-less winters. For a better interpretation of supercooling of studied carabid beetles, more research is required.

Conclusions

Cold adaptation dynamics of carabid beetle species *C. granulatus*, *P. oblongopunctatus* and *P. assimilis* varied seasonally. Carabid beetle species *C. granulatus*, *P. oblongopunctatus* and *P. assimilis* belong to freeze-avoiding cryotype. They are likely to survive unseasonal cold and overwintering could be harmful only in very cold snow-less winters.

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Mõne jooksiklase liigi külmakindluse sesoonne dünaamika: sõmerjooksik (*Carabus granulatus*), metsa-süsijooksik (*Pterostichus oblongopunctatus*) ja süsi-ketasjooksik (*Platynus assimilis*)

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Kokkuvõte

Putukate sesoonne kohastumine sõltub paljudest teguritest, mille hulgas temperatuur on üheks kriitilisemaks faktoriks. Külmakindlus on väga oluline enamike paravöötme putukate talviseks ellujäämiseks ning on inimese seisukohalt ühtviisi tähtis nii kasulikele kui kahjulikele organismidele (Danks, 2007). Jooksiklasi (Coleoptera: Carabidae) esineb liigirikkalt ja arvukalt põllumajandusmaadel ja metsades, kus nad mitmetoiduliste röövmardikadena on taimekahjurite tähtsad looduslikud vaenlased, näiteks söövad jooksiklased nii männikärsakate vastseid kui valmikuid (Salisbury & Leather, 1998; Kromp, 1999; Dillon & Griffin, 2008). Meie kliimavööndis on putukatele võimalike kriitiliste temperatuuride esinemise aeg küllaltki pikk: novembrist märtsini (Paljak, 2007), seetõttu on populatsiooni arvukuse hindamiseks oluline teada nende külmakindlust. Putukate külmakindlust iseloomustab allajahtumisvõime, mille all mõeldakse putuka kudede jahtumist 0 °C madalamas temperatuuris, ilma et sellega kaasneks kudede külmumine (jääkristallide moodustumine). Putuka allajahtumisvõime mõõduks on tema allajahtumispunkt (AJP), s.o. temperatuur milles ta tegelikult külmub ning vabaneb kristallisatsioonisoojus, mis on termoelektriliselt registreeritav (Merivee *et al.*, 1968; Kuusik

et al., 1995; Danks, 2007). Jooksiklased lõpetavad peale talvituskohtadesse jõudmist toitumise, mis võimaldab neil oma allajahtumisvõimet tõsta. Antud eksperimendi eesmärgiks oli määrata kolme jooksiklase liigi – sõmerjooksiku (*C. granulatus*), metsa-süsijooksiku (*P. oblongopunctatus*) ja süsi-ketasjooksiku (*P. assimilis*) külmakindluse sesoonne dünaamika ja külmakindluse strateegia.

Sõmerjooksiku keskmine AJP oli –5.4 °C (jaanuaris), –5.2 °C (mais) ja –6.3 °C (septembris). Metsa-süsijooksiku AJP-d varieerusid –6.3 °C (mais) kuni –7.2 °C (septembris). Mõlema uuritud liigi AJP-d olid sügisel madalamad kui talvel ja kevadel, samas talve ja kevade näitajate vahel erinevus puudus. Süsi-ketasjooksiku AJP oli kõige kõrgem maikuu (–6.8 °C), kolm kuud varem jaanuari oli AJP –8.7 °C. Erinevatel kuudel määratud süsi-ketasjooksiku AJP-d erinesid statistiliselt üksteisest. Uuritud jooksiklaste liigid hukkuvad kui peale AJP käigus eralduvat soojenemist temperatuuri uuesti kuni AJP temperatuurini jahutada, seega on tegemist külmumist vältivate ehk külmamatundlike liikidega (Lee, 1989; Bale, 2002). Tõenäoliselt on katsetatud jooksiklaste liikidele ohtlik allajahtumispunktist madalamate temperatuuridega lumevaene talv.

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