



Supercooling Points (SCPs) of Social Hymenopterans, *Apis mellifera* (Hymenoptera: Apidae) and *Vespa velutina* (Hymenoptera: Vespidae)

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Abstract

Apis mellifera (Hymenoptera: Apidae) is an important pollinator but being threatened by *Vespa velutina* (Hymenoptera: Vespidae), an invasive predator in South Korea. Supercooling point (SCP) is one of indicators of cold tolerance but SCP has not studied for both *A. mellifera* and *V. velutina*. Morphological factors like body traits influence fitness of insects in terms of their thermal tolerance. Therefore, we determined SCPs of honey bee, *A. mellifera* workers, and wasps, *V. velutina* workers and *V. velutina* queens, and analyzed the influence of body traits; body size and body mass on SCPs of these species. This is the first report of SCPs of *V. velutina* queen and worker. The SCPs of *A. mellifera* workers, *V. velutina* workers, and *V. velutina* queens were -6.1 , -6.5 , and -11.3°C , respectively. Both *A. mellifera* and *V. velutina* have unimodal distribution of their SCPs. Neither body size nor body mass influenced the SCPs of *A. mellifera* and *V. velutina*. This study showed that SCPs of social insects, *A. mellifera* and *V. velutina* were much lower than those of solitary overwintering insects, and differed relative to the caste of the same species.

Keywords

Overwinter, Cold tolerance, Caste, Body traits, Invasiveness

INTRODUCTION

Temperature is one of the influential factors for development and reproduction of ectothermic invertebrates such as insects (Noor-ul-Ane and Jung, 2020), affects seasonal and geographical distribution. Ectothermic insects have colonized regions that are seasonally or perpetually cold, and must cope with temperatures that fall substantially below 0°C (Costanzo and Lee, 2013). Survival during the extreme temperature condition is crucial for sustained existence and proliferation (Teets and Denlinger, 2013). For these cold-hardy organisms, capacity for cold tolerance is tuned to the temperatures and exposure durations that a given species encounters within its habitat (Addo-Bediako *et al.*, 2000; Noor-ul-Ane and Jung, 2021). Survival under low temperature depends on either freeze avoidance through supercooling

or freeze tolerance (Sinclair *et al.*, 2003). Supercooling is a metastable state in which body fluids remain liquid below the subzero condition, promoted by increased hemolymph cryoprotectants such as glycerol or other antifreeze proteins which protect against chilling injury or remove inoculative freezing ice-nucleating agents (Duman *et al.*, 1991). Freeze tolerance is the strategy allowing survival even with part of the body frozen, but managing tissue and cell from frost damage. Freeze tolerant insects could initiate the freezing at relatively high temperatures through inoculative freezing by ice nucleating proteins (Sinclair *et al.*, 2003; Jakobs *et al.*, 2015; Teets *et al.*, 2020). In temperate regions of the northern hemisphere where the cold comes seasonally for longer periods, the main strategy is freeze avoidance while in the southern, and freeze tolerance is also more prevalent in insects (reported in 85% of species studied; Sinclair *et*

al., 2003).

Ants, bees, termites and wasps are social insects which constitute most of terrestrial habitats (Holldobler and Wilson, 1990). Social insects not only regulate their own body temperature but also for their nest mates, whereas solitary insects only responsible to regulate their own body temperature (Kadochová and Frouz, 2013). Honey bees, plant pollinators, are well known for their contribution in the yield production of the different crops (Jung, 2008; Begna *et al.*, 2020). Winter survival is a challenging aspect of *Apis mellifera* in temperate regions. *A. mellifera* survives in winter by forming a cluster and maintaining temperature around 20°C (Southwick, 1985). Cold temperature may challenge *A. mellifera* workers during autumn for their foraging and defense purpose. Cold tolerance of sub-species of honey bees has been studied (Li *et al.*, 2012; Qin *et al.*, 2019) but there is no report from Korea. *Vespa velutina nigrithorax*, an invasive predator of honey bees, has been established in South Korea since 2003 (Park and Jung, 2016). All casts of *V. velutina* dies in winter except mated queens which overwinters in the soil or tree crevices (Monceau *et al.*, 2014) but there is no report of cold tolerance of *V. velutina*.

Body size is also another fitness trait in organisms including insects, usually larger individual benefitted more in terms of their survival, fecundity and mating success (Honek, 1993; Andersson, 1994) subsequently contributing for ecosystem functioning (Enquist *et al.*, 2003). Stress resistance is also more evident in larger insects (Hood and Tschinkel, 1990). Larger insects show more desiccation resistance and foraging time (Kaspari, 1993) within and between species (Chown *et al.*, 1999). Starvation is also another stress which helps larger insects for their survival as they have more energy reservoirs or lower metabolic activities (Harshman and Schmid, 1998). Body size also plays an important role in thermoregulation of organisms (Schmidt-Nielsen, 1997) including insects both in cold tolerance and heat tolerance (Baudier *et al.*, 2008; Shepherd *et al.*, 2008). Similarly, body mass is also important for ecosystem functioning, as it controls energy uptake and transformation in ectotherms at organismal level and it also link to stress resistance including thermal tolerance of insects (Oyen *et al.*, 2016; Knapp and Řeřicha, 2020).

Underlying the importance of cold tolerance for these

species, we determined Supercooling points of *A. mellifera* workers and *V. velutina* queen and also determined influence of body size and body mass on SCP within and across these species and then estimated SCPs compared with SCPs of social hymenopterans.

MATERIALS AND METHODS

1. Source of insect

Worker of *A. mellifera* were collected from winter clusters from beekeepers of Andong city, (36°33' N 128°44' E), Gyeongsangbuk-do, South Korea during first week of December, 2020 and maintained (@15 bees/cup) in plastic cups (bottom dia. 9 cm × top dia. 12 cm × height 8 cm, with side ventilation slots), at 25 ± 1°C, 35 ± 5% RH, under total dark condition in an incubator until use in experiment. Pollen patty was provided as a diet for the *A. mellifera*. Pollen patty diet was composed of rape seed pollen, sugar, soybean flour, yeast and nutrient supplement in 20 : 60 : 20 : 20 : 1 ratio by mass. Adults were also provided 50% sugar-water solution by volume soaked in cotton.

Workers and queens of *V. velutina* were collected on first week of December, 2020 from vespiculture (a semi-field rearing screen house, established at Andong National University, Andong, Gyeongsangbuk-do, South Korea), identified by mesoscutum width (MW) (Pérez-de-Heredia *et al.*, 2017) and maintained individually in above mentioned plastic cups at 4 ± 1°C, 35 ± 5% RH, provided with 50% sugar-water solution under total dark condition in an incubator until used in experiment.

2. Supercooling point determination

We determined SCPs of 17 workers of *A. mellifera*, and 11 workers and 12 queens of *V. velutina*. Workers and queens were attached with type-T copper thermocouple (BTM-4208SD, LT Lutron, Taipei, Taiwan) placed individually into 1.5 mL microcentrifuge tubes. Temperature was recorded at 1 second interval. The thermocouple was passed through the bottom of tube by making small hole and attached with body of insects. Four PCR tubes containing individual worker and queen were put in a styrofoam box (30 × 30 × 15 cm), which was then placed in a refrigerator at -40°C until the thermo-

couples reached a temperature of -30°C . The cooling rate was measured as $0.6^{\circ}\text{C}/\text{minute}$. Body length was measured by digital caliper and body mass of both species were measured with an electronic balance (0.001 g) (AINSWORTH, US/Model:10) before being used for SCP determination.

3. Statistical Analysis

One-way ANOVA was applied to find out the difference between SCPs of *A. mellifera* workers, *V. velutina* workers and *V. velutina* queens. Linear regression was carried out to establish relationship of body length and body mass with SCP. All the analyses were conducted using SPSS v20 software.

RESULTS AND DISCUSSION

The SCPs of *A. mellifera* and *V. velutina* workers were ranged from -3.5 to -8.2°C and -4.2 to -8.8°C , respectively, with a median value of -6.2 and -6.5°C , respectively (Fig. 1). The SCP of *V. velutina* queen was $-11.3 \pm 0.8^{\circ}\text{C}$ and significantly higher than values of *A. mellifera* workers and *V. velutina* (ANOVA, $F=74.5$, $df=2,37$, $P>0.001$). Histogram shows that SCPs of both *A. mellifera*, *V. velutina* queen, and *V. velutina* workers have unimodal distribution (Fig. 2). SCP of *A. mellifera* of present study is almost similar to its subspecies of *A. mellifera ligustica* which has SCP of -5.3°C calculated by Li *et al.* (2012) and -6.4°C in December collected workers in another study of Qin *et al.* (2019). Even

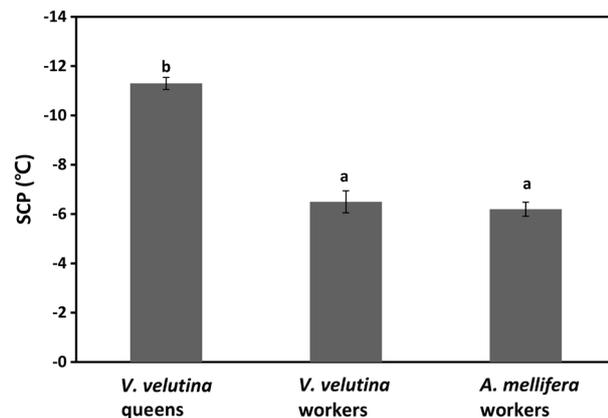


Fig. 1. Supercooling points of *A. mellifera* workers, *V. velutina* workers and *V. velutina* queens.

though winter honey bees are fully equipped with fat body and higher density of hemolymph proteins, SCPs of winter honey bees were relatively higher since the honey bees overwinter in the winter cluster within the hive in the protected space (Southwick, 1985). But still honey bees are risky and susceptible for freeze dying if winter cluster being broken or exposed to the cold even relatively shorter time associated with parasitic mites or disease (van Dooremalen *et al.*, 2018).

The SCP of *V. velutina* worker from South Korean population was -6.5°C which is almost similar to some of other hymenopteran workers of *Polistes annularis* (-6.8°C) and *Bombus terrestris* (-7.1°C) (Strassman *et al.*, 1984; Owen *et al.*, 2016). However, SCP of *V. velutina* queen (-11.3°C), was higher than SCPs of worker *V. velutina* ($F=74.5$, $df=2,37$, $P>0.001$), also close to SCPs of *P. annularis*, -12.9°C and *Sirex noc-*

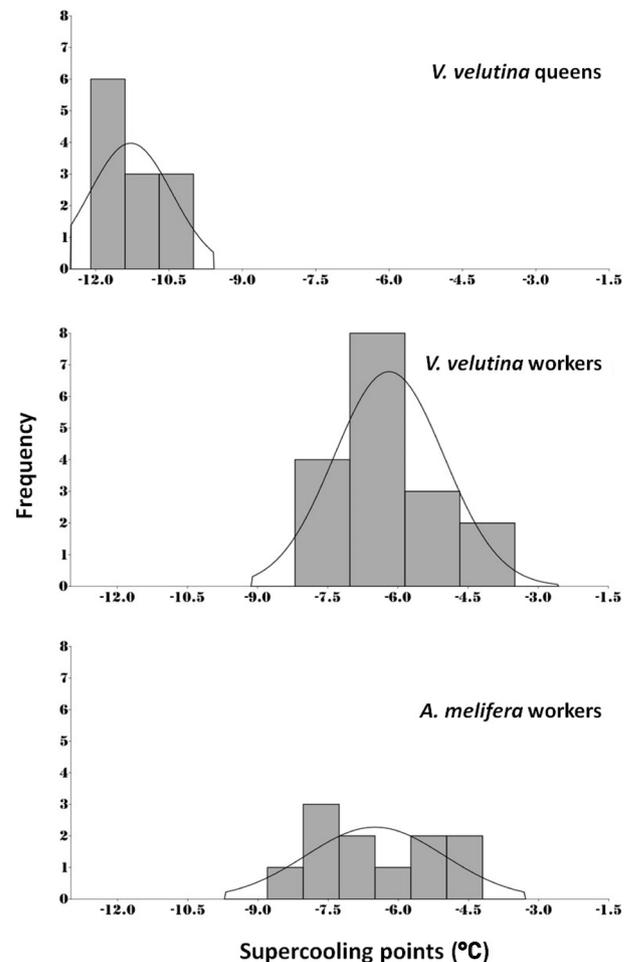


Fig. 2. Frequency distribution the Supercooling points of *A. mellifera* and *V. velutina*.

tilio, -11.8°C (Strassman *et al.*, 1984; Li *et al.*, 2019). As queens of *V. velutina* should overwinter, but not workers, and establish new colonies in the next spring (Monceau *et al.*, 2014), there are physiological differences between workers and queens and that fat body appearance is also a reliable indicator of caste. For *P. exclamans*, 83% of the future queens survived at the lower conditioning temperature, $+5^{\circ}\text{C}$ of 15 days exposure while 24% of the workers survived during the same exposure.

SCPs were not influenced by the body size and body mass for both *A. mellifera* ($F=1.5$, $df=1,19$, $P=0.2354$), and *V. velutina* worker ($F=0.51$, $df=1,19$, $P=0.4919$), and *V. velutina* queen ($F=0.16$, $df=1,10$, $P=0.69554$) (Fig. 3). Similarly, body length also did not influence SCPs of both *A. mellifera* ($F=1.04$, $df=1,19$, $P=0.3217$), *V. velutina* worker ($F=0.23$, $df=1,11$, $P=0.6437$), and *V. velutina* queen ($F=2.70$, $df=1,10$, $P=0.1317$) (Fig. 4). There are some observations which showed that body trait such as body size is linked with SCP (Zachariassen, 1985). Larger body size increased supercooling point in some insects (Sinclair and Chown, 2005). While, no differences were also reported (Knapp and Uhnava, 2014; Yunik and Chilton, 2021). Yunik and Chilton (2021) reported that the different size and weight of male and female ticks showed no difference of SCPs. We found difference in SCPs between different casts of overwintering queen and non-overwintering worker of *Vespa velutina* but no difference in SCPs of individuals in the same cast (worker or queen) with different body size. Also overwintering honey bee worker and

non-overwintering vespa worker which differ in body size, showed similar SCPs, suggesting workers of *V.*

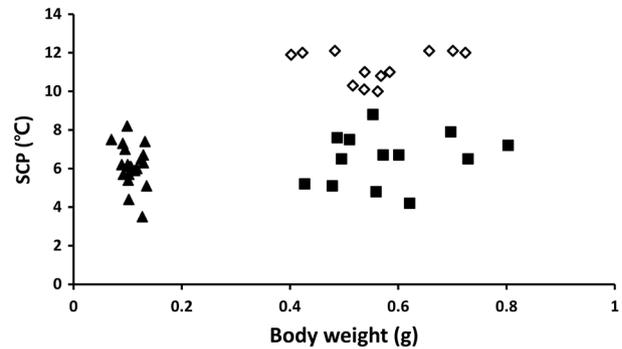


Fig. 3. Supercooling points of *A. mellifera* and *V. velutina* of different body weights. \diamond : *V. velutina* queens ($Y=1.0685x + 10.687$, $R^2=0.0160$), \blacktriangle : *V. velutina* workers ($Y=2.5866x + 5.0168$, $R^2=0.0439$) and \blacksquare : *A. mellifera* workers ($Y=-16.601x + 7.9167$, $R^2=0.0733$).

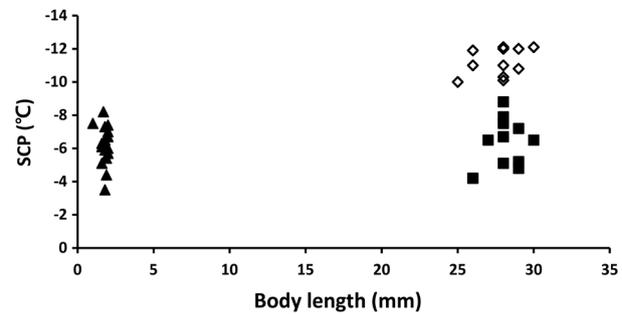


Fig. 4. Supercooling points of *A. mellifera* and *V. velutina* of different body lengths. \diamond : *V. velutina* queens ($Y=0.2483x + 4.3517$, $R^2=0.2123$), \blacktriangle : *V. velutina* workers ($Y=0.1941x + 1.0513$, $R^2=0.0201$), and \blacksquare : *A. mellifera* workers ($Y=-1.0337x + 7.9938$, $R^2=0.0517$).

Table 1. Supercooling points of some hymenopteran species

Insect	Order	Family	Stage	SCP	Reference
<i>Apis mellifera</i>	Hymenoptera	Apidae	Worker	-6.2	Present study
<i>Vespa valutina</i>	Hymenoptera	Vespidae	Worker	-6.5	Present study
<i>Vespa valutina</i>	Hymenoptera	Vespidae	Queen	-11.3	Present study
<i>Apis mellifera ligustica</i>	Hymenoptera	Apidae	Worker	-5.25	Li <i>et al.</i> , 2012
<i>Bombus terrestris</i>	Hymenoptera	Apidae	Worker	-7.1	Owen <i>et al.</i> , 2013
<i>Dolichovespula maculate</i>	Hymenoptera	Vespidae	Gyne	-20.1	Corrigan and Irwin, 2009
<i>Osmia cornuta</i>	Hymenoptera	Megachilidae	Worker	-30*	Krunić and Stanislavljević 2006
<i>Osmia rufa</i>	Hymenoptera	Megachilidae	Adult	-31*	Krunić and Stanislavljević 2006
<i>Camponotus herculeanus</i>	Hymenoptera	Formicidae	Imago	-40*	Berman <i>et al.</i> , 2017
<i>Camponotus obscuripes</i>	Hymenoptera	Formicidae	worker	-8.5	Ohyama and Asahina, 1972
<i>Myrmica kamtschatica</i>	Hymenoptera	Formicidae	Worker	-12.7*	Berman <i>et al.</i> , 2012

*only lowest SCP was selected.

velutina and *A. mellifera* may have similar cold tolerance. The study suggested that larger insects do not necessarily have higher SCPs.

There are numerous factors influencing SCPs; cryoprotectant level (Somme, 1982), developmental stage (Bouchard *et al.*, 2006), feeding (Cannon, 1986) apart from body size. The workers of ant, *Coptotermes herculeanus*, have the lowest SCP (-40°C) followed by a mason bee, *Osmia cornuta*, (-31°C) (Table 1). The highest SCP of value -4.3 and -6.1°C , belongs to *Myrmica angulinodis* and *A. mellifera*, respectively. The queen of paper wasp, "*Polistes anularis*" showed SCP of value -6.8°C . While, the queens of *Vespa pensylvanica* showed lower SCP values (-23.3°C). In conclusion, present study shows that SCP of *V. velutina* queen is lower than *V. velutina* workers and *A. mellifera* workers. Body length and body mass did not have any influence on SCP of within and across species of *A. mellifera*, *V. velutina* queen, and *V. velutina* workers.

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