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Body Compositional Changes of Fatty Acid and Amino Acid from the Queen of Bumblebee, *Bombus terrestris* during Overwintering

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Abstract

The body weight and body compositions were measured on Buff-tailed Bumblebee, *Bombus terrestris* queen during overwintering to understand the change of the physical status and to evaluate the nutritional requirement for the oviposition by queen after overwinter. Results revealed increase in body weight and body fat (6.5 and 9.5% respectively) during the first week of overwintering initiation and subsequent decrease (11 and 79.5% respectively) in the later phase of overwintering. On the other hand, during early stage of overwinter the total body protein content increased by 12.3%, and by the end of overwintering, it was 37.6% higher than that of early stage. In the present investigation, the decreasing trend of fats and increasing trend of amino acids and thus proteins during overwintering suggest that the fats are primarily utilized for the metabolic purposes during diapause when bumble bee queens could not consume any feed, and the protein helps to sustain and lowering the freezing point in time of sub-zero temperatures of overwintering in temperate zone.

Key words: Metabolism, Energy consumption, Nutritional requirement, Fat, Annual nest

INTRODUCTION

Native pollinators often plays essential role in pollination of wild and cultivated plants in all terrestrial ecosystems (Garibaldi *et al*., 2013). Bumblebees, in particular are among the most important pollinators of temperate zone plants (Inari *et al*., 2012). The dense hair on their bodies allows efficient pollen transfer from flower to flower. Moreover, they also exhibit a distinctive feature of sonication often helps in pollination called 'buzz pollination'. Although bumblebees are efficient pollinators of a variety of crops including red clover, cranberries, blueberries, kiwi, almonds, apples, pears etc. but majority of the commercially reared bumblebee colonies are used in production of greenhouse tomatoes and sweet pepper (NAPPC, 2006).

In the year 1985, de Jonghe, the Belgian veterinarian and amateur bumblebee researcher first uncovered the economic benefits of using bumblebee for pollination of greenhouse tomatoes and subsequently founded the commercial rearing of *Bombus terrestris* company Biobest in 1987 (Velthuis and van Doorn, 2006). Couple of commercial production facilities namely Koppert Biological Systems and Bunting Brinkman Bees were initiated in the next two years. Resulting from the international trade millions of the colonies shipped throughout the world leading to deliberate releases or accidental escapes that foster *B. terrestris* establishments (Goulson, 2010; Inari *et*

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al., 2005; Murray *et al*., 2013; Velthuis and van Doorn, 2006; Lococq *et al*., 2016).

In general, in temperate region bumblebees are univoltine i.e. they have only one generation per year. However, observations reported that second generation could exist for *B. jonellus* in field (*cf*. Röseler, 1985; Meidell, 1968). New queen emerges at the end of the season. Young queen mates with multiple drones and stores sperms for the rest part of her life. These young queens are the only members of the colony who survive through winter. They do so by adapting a strategy called 'diapause' which can be defined as resting state of their life cycle. Diapause is a suspension of development that can occur at the embryonic, larval, pupal, or adult stage depending on the species. Entering into diapause is an endogenous characteristic for queen caste. Young queens participate in the process often known as 'overwintering'. Depending on the species diapause can be facultative or obligatory. In case of bumblebee *B. terrestris* diapause is an obligatory part of their life cycle.

Young queens prepare themselves for entering into the diapause phase (a form of hibernation). They consume food and synthesize the reserves for diapause phase of life since the first day of eclosion. About on third day they become ready to mate. After copulation the queens become lazy, seek a suitable site for overwintering and enter diapause independent of temperature or light (Röseler, 1985). In captivity under environment controlled condition queen sit apart from the nest and do not participate any household tasks. Low juvenile hormone titre in haemolymph resulting from the inactivation of neurosecretory cells controlling corpora allata, accompanied by other physiological changes like deposition of fat and other metabolites in fat body but not in ovaries are the causes of imaginal or reproductive diapause (Röseler, 1976; 1977; Brown and Chippendale, 1978; Saunders *et al*., 2002). However, hardly any study is available to demonstrate the changes in body composition during the initiation and termination of overwinter in *B. terrestris*. An understanding such changes are fundamental in clarifying the sequences of the physiological events which would be of a great value in formulating diet for them.

MATERIALS AND METHODS

Bumblebee collection

Young queens of *B. terrestris* of three different physiological conditions viz. mated (n=19), after one week of overwinter (early stage) (n=20) and at the end of overwinter for 3 months (n=20) were obtained from Yecheon Entomology Institute, Republic of Korea. *B. terrestris* colonies are reared in captivity under controlled climatic condition at +28°C to +30°C and relative humidity is maintained 60%.

Body weight measurement

B. terrestris queens were narcotized by applying carbon di oxide (CO_2) and body weight of individual was obtained by electronic balance. One way ANOVA and Tukey's HSD test were performed in order to analyse difference in the body weight of queens of different physiologic conditions using SAS 9.2.

Sample preparation for chemical analyses

The queens were freeze dried for 72 hours at −50°C using the freeze dryer (Christ Alpha1-4 LD Plus, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) and ground to powder form and used for further analysis. All the chemicals used in the study were of analytical grand and glass wares were meticulously clean.

Amino acid analysis

Amino acid composition was determined by S433 (Sykam GAmbH, Gemany) equipped with LCA K07/Li (PEEK-column 4.6 X 150mm) column following the standard method of AOAC (Association of Official Analytical Chemists) (1990). The powder samples were hydrolysed in 6N hydrochloric acid (HCl) at 110°C for 24 hours under nitrogen environment followed by reconstitution with dilution buffer (0.12N, pH 2.20) and injected in the analyser.

Fatty acid analysis

Fatty acid composition was analysed by Gas chromatography (GC-14B, Shimadzu, Tokyo, Japan) equipped with Flame Ionization Detector (GC-FID) and SP-2560 column following the standard method of Korean Food Standard Codex (2010). The samples were derived into fatty methyl ester (FAMEs) and injected into GC.

RESULTS AND DISCUSSION

During diapause *B. terrestris* queens do not consume any food. It implies that they must sequester sufficient nutritional reserve in the pre-diapause period to meet its metabolic needs during diapause and still have sufficient reserve remaining at the end of diapause to resume normal activity. Fig. 1 demonstrates the changes in body weight of mated *B. terrestris* queen, one week from the overwintering and at the end of overwinter process. There is a significant changed in the body weight of *B. terrestris* in different physiological conditions (dF=2,56; F=10.86; $P<0.000$). Firstly the body weight was found to be increased by 6.4% in the time of one week after the initiaiton of overwintering. After the young queens emerge, each queen mate with multiple drones and store sperms for the rest of their life. Few days after mating they go for the diapause

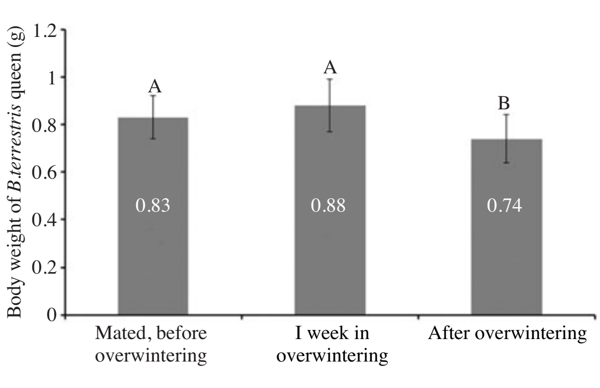

Fig. 1. Body weight (g) of mated, one week from the initiation of overwinter and overwintered *Bombus terrestris* queen.

which is a programmed strategy to survive in the winter. In the mean time they eat more and prepare themselves for the diapause by reserving the food which could be utilized during this period. Presumably this is the cause that the body weight was increased during the first few days when overwintering was induced. In time of termination we found the body weight was decreased almost 11% comparing to the mated *B. terrestris* which was found statistically significant (Fig.1).

To understand the role of fat during the overwinter period we analysed the fatty acid composition of the fat fraction of the *B. terrestris*. Table 1 represents the fatty acid compositions of mated *B. terrestris* queen, after one week from the initiation of overwinter and at the termination of overwinter process. Oleic, Palmitoleic, palmitic, myristic acid were found predominated among the fatty acids. The fatty acid reflects the similar pattern observed for the body weight of *B. terrestris* queens. Total body fat was increased by 9.5% during the first week of the overwinter and decreases rapidly 79.5% during the latter part of overwintering. The pattern suggests that overwintering *B. terrestris* queens utilize fat stores as the primary metabolic substrate.

Irrespective of the physiologic situations body fat of *B. terrestris* queens mostly comprises of monounsaturated fatty acids (76.2~76.7%). In general, monounsaturated fatty acids (MUFA) was followed by saturated fatty acids (SFA) and less concentration was found for polyunsaturated fatty acids (PUFA) in *B. terrestris* which is in agreement with other scientific reports on different insect species (Bhulaidok *et al*., 2010; Chakravorty *et al*., 2011; Rumpold and Schlüter, 2013; Chakravorty *et al*., 2016; Ghosh *et al*., 2017). However, the utilization of different categories of fatty acids differed significantly. SFA and MUFA in overwintered *B. terrestris* queen were decreased by 83.8 and 79.3% respectively while comparing with the body mated in time of diapause initiation i.e. mated queens. However, the PUFA proportion was decreased only by 42.5%. Fatty acids serve various functions during whole life cycle of insects including biosynthesis of wax, pheromones, sex pheromones, prostaglandins and other

Table 1. Fatty acid composition (mg/100g Dry matter basis) of mated, one week from the induction of overwinter and overwintered *Bombus terrestris* queen

	Mated		1 week overwintered		Overwintered	
	B. terrestris		*B. terrestris*		*B. terrestris*	
	mg	%	mg	%	mg	%
Lauric acid (C12:0)	66.40	0.79	84.10	0.91	4.46	0.26
Tridecanoic acid (C13:0)	ND	ND	10.57	0.11	3.42	0.20
Myristic acid (C14:0)	324.36	3.84	382.83	4.14	27.65	1.60
Palmitic acid (C16:0)	1287.10	15.25	1500.7	16.24	155.63	8.98
Stearic acid (C18:0)	140.94	1.67	167.83	1.82	89.32	5.15
Arachidic acid (C20:0)	ND	--	ND	--	13.68	0.79
SFA (Subtotal)	1818.80	21.55	2146.03	23.22	294.16	16.97
Myristoleic acid (C14:1)	129.22	1.53	128.49	1.39	3.50	0.20
Palmitoleic acid (C16:1)	1393.58	16.51	1479.80	16.01	72.45	4.18
Oleic acid (C18:1 n9 cis)	4315.18	51.13	4656.66	50.39	899.12	51.87
Cis-11 Eicosenoic acid (C20:1)	594.58	7.04	630.79	6.83	354.60	20.46
MUFA (Subtotal)	6432.56	76.21	6895.74	74.62	1329.67	76.71
Linoleic acid (C18:2 n6 cis)	188.96	2.2	199.23	2.16	108.58	6.26
PUFA (Subtotal)	188.96	2.14	199.23	2.16	108.58	6.26
TOTAL	8440.32		9241.00		1733.39	

* ND = Not Determined

Table 2. Amino acid composition (g/100g Dry matter basis) of mated, one week from the induction of overwinter and overwintered *Bombus terrestris* queen

	Mated		1 week overwintered		Overwintered	
	B. terrestris		*B. terrestris*		*B. terrestris*	
	g	%	g	%	g	%
Valine	2.36	6.16	2.79	6.48	3.96	6.68
Isoleucine	1.85	4.83	2.36	5.48	3.42	5.77
Leucine	3.07	8.01	3.89	9.04	5.67	9.57
Lysine	3.03	7.91	2.89	6.71	3.81	6.43
Threonine	0.9	2.35	0.84	1.95	1.2	2.03
Phenylalanine	1.17	3.05	1.28	2.97	1.84	3.11
Arginine	1.88	4.91	2.13	4.95	2.73	4.61
Methionine	0.32	0.84	0.39	0.91	0.71	1.20
Histidine	1.02	2.66	1.21	2.81	1.75	2.95
Tyrosine	1.16	3.02	1.4	3.25	2.04	3.44
Aspartic acid	1.46	3.81	1.74	4.04	2.47	4.17
Glutamic acid	4.84	12.63	5.33	12.38	7.22	12.19
Serine	2.37	6.18	2.1	4.88	2.94	4.96
Glycine	3.09	8.06	3.53	8.20	5.01	8.46
Alanine	3.94	10.28	4.47	10.38	6.12	10.33
Cystine	2.05	5.35	2.34	5.44	3.07	5.18
Proline	3.81	9.94	4.36	10.13	5.29	8.93
TOTAL	38.32	100	43.05	100	59.25	100

derivative of PUFA, defensive secretions etc. to mention a few (Stanley-Samuelson *et al*., 1988). During nonfeeding period like diapause they play role as the primary energy sources. Although ample reports are available on fatty acid composition of many different species of insects, but study on changes in fatty acid composition attending have been addressed in few species and mostly restricted to comparative account of diapause and non-diapause states

(Khani *et al*., 2007). *Dolycoris baccarum* (Hemiptera) and *piezodorus lituratus* (Hempitera) diapausing adult had different fatty acid composition in diapausing and prediapausing condition. Diapausing adult was reported to contain higher proportion of MUFAand lower proportion of SFA (Bashan and Cakmak, 2005). Unsaturated fatty acid/saturated fatty acids ratio (UFA/SFA) increased in total phospholipid in overwintering larvae of *Eurosta solidaginis* (Diptera) (Bennett *et al*., 1999). In the present study, UFA/SFA ration was increased from 3.6 to 4.9 in overwintering *B. terrestris* queen which is also in agreement few other insect species (Khani *et al*., 2007). Linoleic acid is a structural component of membrane to maintain peoper fluidity and permeability. Presumably, the increased proportion of unsaturated fatty acids could be attributed to the adaptation to cold in order to maintain appropriate fluidity of the food reserve to make them available as energy source (*cf*. Khani *et al*., 2007).

Many insect species like larvae of *Peiris rapae* (Lepidoptera), larvae of *Plodia interpunctella* (Lepidoptera), last instar larvae of *Pectinophora gossypiella* (Lepidoptera), *Culex pipens* (Diptera) show diapause-associated increases in reserves or body size and thus body weight (*cf*. Hahn and Denlinger, 2007; Kono, 1970; Tsuji, 1958; Adkisson *et al*., 1963; Mitchell and Briegel, 1989). Present investigation demonstrated that the *B. terrestris* queen belongs to this group and it also supplements previous scientific report by Fliszkiewicz and Wilkaniec (2007) in which the diapausing *B. terrestris* queens were characterized to contain higher dry matter in fat body and higher fat content as compared with their non-diapausing counterparts. It makes sense for diapausing individuals to increase their reserves as a strategy to deal with the energetic demands of the diapause period. Lipids are primary metabolic reserve. The trend of reducing body fat level during overwinter period is found similar to the study on parasitoid wasp *Asobara tabida* (Hymenoptera) in which the fat reserve and also fecundity decreased significantly with the time spent in diapause (Ellers and van Alphen, 2002). During diapause insect could not obtain water which could be generated from lipids and help insects in survival (*cf*. Yocum *et al*., 2011; Wharton, 1985; Danks, 2000). This could be one possible use of fat. In fact, the metabolic pathways leading to cell proliferation and growth are down regulated during diapause while basic cellular maintenance remain operational at reduced rate and stress resistance pathways leading to cryoprotectant and heat shock protein (HSP) synthesis etc. are likely to be up regulated (Dehlinger *et al*., 2005). However, on the other hand accumulation of greater reserve increases the risk of attracting natural enemies or not completing development before the onset of inclement condition (Masaki, 1977; Tauber *et al*., 1986). Presumably this could be a possible reason few species do not follow this trend. To cite few examples, diapausing larvae of *Calliphora vicina* (Diptera), diapausing pupae of *Meduca sexta* (Lepidoptera) do not show any significant greater store of fat or nutrients than their non diapausing counterpart (Saunders, 1997; 2000; Siegert, 1986). Diapausing pupae of *Papilio polyxenes* (Lepidoptera), larvae of *Choristoneura fumiferana* (Lepidoptera) are smaller than their non diapausing counterparts (Blau, 1981; Harvey, 1961).

Table 2 represents the amino acid content of mated *B. terrestris* queen, one week from the initiation of overwinter and at the termination of overwinter process. In contrast to the pattern of changes in fatty acids, a pronounced increase took place during overwintering period. During early stage of overwinter initiation i.e. one week after the initiation of overwinter the protein concentration was raised by 12.3% from the mated queen and subsequently it was increased by 37.6%.

Similar kind of observation was reported for *Pectinophora gossypiella* (Lpidoptera). Concentration of total protein was considerably increased by 62.3% in haemolymph during diapause though a drop occurred in fat body protein concentration in late phase of diapause (Rostom *et al*., 1992). Post-feeding larvae of *Diatraea grandiosella* (Lepidoptera) and diapausing adult female of *Leptinotarsa decemlineata* (Coleoptera) accumulate markedly greater quantity of hexamerin protein (Brown and Chippendale, 1978; de Kort and Koopmanschap, 1994). Rise in protein might add to the concentration of solutes, which in turn, lower the freezing point of the haemolymph, that enables the species to withstand unfavourable low temperature during diapause. Prediapausing larvae of *Diatraea grandiosella* were found to store protein fraction which makes up 20% of the soluble proteins of the fat body of the newly diapaused individuals (Brown and Chippendale, 1978). Moreover, in the diapause associated protein of *D. grandiosella* aspartate, leucine, lysine and glutamate was found predominate (Brown and Chippendale, 1978). In the present study also, *B. terrestris* queen contained these amino acids in higher proportion. In addition higher proportion of alanine and glycine were found (Table 2). In Colorado potato beetle *L. decemlineata* protein accumulates first in haemolymph and later in fat body often known as 'diapause protein' which acts as a storage protein. The other blood proteins also increase under short day, resulting almost 3 times higher concentration of total proteins in haemolymph and remains high during diapause (*cf*. de Kort, 1990; de Loof and de Wilde, 1970).

In the present investigation the decreasing trend of fats and increasing trend of amino acids and thus proteins during overwintering suggest that the fat primarily are utilized for the metabolic purposes as they could not feed at that time and on the other hand the protein helps to sustain in time of sub-zero temperature by lowering the freezing point. Thus, high fat diet could be supplied to these overwintered queens in captivity.

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