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Utilization of lipid for flight and reproduction in *Spodoptera litura* (Lepidoptera: Noctuidae)

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Abstract. Research of the physiological traits of a long-distance migratory insect, the common cutworm *Spodoptera litura,* in relation to flight and reproduction, was focused on triacylglycerol (TG) levels and their fatty acid composition.

Tethered flight experiments demonstrated that three-day old male moths can fly more than 20 h. Among eight identified fatty acids of which the adult TG is composed, the ratios of the following unsaturated fatty acids, palmitoleic acid (C 16:1), oleic acid (C 18:1), linoleic acid (C 18:2) and linolenic acid (C 18:3), gradually declined with longer flight duration. On the other hand, the TG levels of non-flown males reared for ten days on only water, were the same as those of three-day old males after 12 hr of flight, but the ratios of these unsaturated fatty acids in TG's remained nearly unchanged. These results suggest that the unsaturated fatty acids in TG are mainly used as a flight energy source.

As ovarian development in females of this species occurs shortly after adult emergence, long-distance migratory capacity has been regarded as highly unlikely. In fact, ovarian development was completed within three days after adult emergence and females laid eggs thereafter. During this three day period TG increased in the ovary, accompanied by increasing ratios of unsaturated fatty acids in the TG's and the ovary eventually occupied most of the abdomen, in spite of a nearly constant level of TG in the abdomen and unchanged ratio of its unsaturated fatty acids. These facts support the transfer of TG from the fat body to the ovary, and further suggest that the mature females utilize the TG deposited in the ovaries as a flight energy source. Then if they migrate a long distance, they use residual TG after flight for egg production.

INTRODUCTION

Long-distance migratory moths use mainly sugar during initial flight as an energy source, and then lipid deposited in the fat body during longer flight (Beenakers et al., 1985). Once they begin flight, adipokinetic hormone is released from the corpora cardiaca and triggers diacylglycerol (DG) mobilization from the triacylglycerol (TG) that is stored in the fat body. DG is transported to flight muscle and the lipid constituent, fatty acid, is oxidized and used as flight fuel (Blacklock & Ryan, 1994).

The common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae), is a serious pest in many vegetables and crops in western Japan and Southeast Asia. They do not enter diapause in any of the developmental stages (Miyashita, 1971). In western Japan, the larvae are sometimes observed in fields by early summer, but generally outbreaks occur near autumn. We demonstrated coincidental increases in male catches by pheromone traps in different areas 300 km apart in southen Japan at the time of a typhoon and proposed that they immigrated to Japan with the aid of typhoon winds (Murata et al., 1998). Evidence of long-distance migration by this species comes from observation of male moths over the East China Sea and the Pacific Ocean (Kiritani, 1984). On the other hand, long-distance migratory capacity has been regarded as highly unlikely, as ovarian development in females of this species occurs within three days after adult emergence (Okamoto & Okada, 1968). Although physiological traits of *S. litura* adults have been compared with regard to tethered flight and the differences between sexes and with aging (Noda & Kamano, 1988; Saito, 2000), no work has been reported on the energy sources for long flight.

The purpose of this report was to assess the long-distance migratory capacity of *S. litura* from the TG levels and their fatty acid composition after flight. Here, we first compared the flight ability of male moths by tethered flight and investigated the residual amount of TG and its fatty acid composition in flown and non-flown moths. We then analyzed the amount of TG and its fatty acid composition in both ovary and abdomen during ovarian development in females.

MATERIALS AND METHODS

Insects

Eggs of *S. litura* were supplied from Sumitomo Chemical Co. Ltd., where these animals have been reared for more than 20 years. The larvae were reared at 25 ± 2°C ? under 16 h light - 8 h dark photo-regime on an artificial diet, composed mainly of kidney bean, yeast powder and wheat germ (modified from Okamoto & Okada, 1968). The pupae were sexed based on morphology of the abdominal terminal segments, and males and females were kept separately after three days of pupation. The adults were collected within 8h of adult emergence, and were kept individually in 10 ml test tubes containing a segment of filter paper moistened with water as a foothold and also to prevent unnecessary movement of flight muscle and consumption of flight energy. Adult age on the day of emergence was designated as 0. Three-day old male moths were used for tethered

TABLE 1. Comparison of the percentage of fatty acid composition in TG's and the amount of TG in the abdomens of flown *S. litura* males. 1)

Fatty acid (%)	12 : 0	14 : 0	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	18 : 3	Other	Saturated	Unsaturated	Level of TG (μmol/g.d.w.) 2)
Flight duration (hr)												
0	0.3	0.8	44.8	9.9	1.2	28.7	12.5	1.8	0.0	47.1	52.9	849.7 ± 185.4
1	0.6	0.6	50.6	6.4	1.1	29.3	10.1	1.3	0.0	52.9	47.1	945.4 ± 188.4
3	1.2	1.0	49.4	7.5	1.3	27.1	11.1	1.4	0.0	52.9	47.1	831.1 ± 55.5
6	0.4	1.9	55.0	5.7	2.9	23.0	9.1	0.8	1.2	60.2	38.6	715.0 ± 235.8
12	12.0	5.7	50.8	3.8	4.8	17.0	5.5	0.4	0.0	73.3	26.7	510.4 ± 418.9
15	13.9	8.9	54.3	3.6	1.7	13.3	4.0	0.3	0.0	78.8	21.1	408.9 ± 241.9
> 18	15.1	11.4	50.9	3.1	9.2	4.3	1.2	0.0	4.8	86.6	8.6	144.3 ± 147.8

1) Three days-old adults were flown by tethered flight.
2) Values are averages with S.D. (n = 5–8).

flight, when the highest flight activity was observed (data not shown). After flight, the abdomens were dissected and kept at -20°C in a freezer until lipid analysis was performed. As controls, non-flown male moths were separately maintained in test tubes with only water, and sampled at 0, 3, 7 and 10 days after emergence without being used for tethered flight. Abdomens from these insects were dissected and were kept in the freezer. The wet weight of ovaries attained a maximum within three days of adult emergence, after which some females started to oviposit; three-day old females were therefore judged as reproductively mature. To investigate the possible transport of lipid to ovaries from other parts of the body, abdomens (including ovaries) and ovaries dissected from different individuals (n = 5) were prepared from 0-day and 3-day old females, and were frozen for lipid analysis.

Lipid analysis

Total lipid was extracted according to Folch et al. (1957). Lyophilized abdomens or ovaries were weighed and then homogenized in chloroform-methanol (2:1, v/v), and the homogenate was incubated at 37°C for 30 minutes with shaking. After being cooled, the homogenate was diluted to 25 ml with chloroform-methanol (2:1, v/v) and filtered through filter paper (Whatman No.2). The filtrate was added to 4.7 ml of distilled water and mixed with gentle shaking. After the mixture was placed at 4°C overnight, the chloroform layer was collected and dried under nitrogen, and lipid fractionation was carried out by thin-layer chromatography (TLC) according to Wada & Sugano (1972). The residues obtained in the above procedure were dissolved in 2 ml of petroleum ether, and 40 μl were spotted onto a silica gel G plate (Whatman K5, 20 cm × 20 cm), which had been activated 2 h at 110°C, and separated with petroleum ether-diethyl ether-acetic acid (82:18:1, v/v/v). The TLC plate was dried under nitrogen and the separated components visualised under iodine vapor. After addition of an internal standard (pentadecanoic acid, Sigma Chemical Co.) to the TG fraction cut from the plate, the fatty acids in it were converted to their methyl esters by refluxing in 17% (v/v) oxygen-free hydrochloric acid in methanol at 65°C for 3 h. The methyl esters were then extracted twice with 2 ml of hexane from the hydrochloric acid/methanol fraction. Fatty acid composition of TG was determined by gas-liquid chromatography (GLC, Shimazu GC-14B, Kyoto, Japan) with a hydrogen flame ionization detector and a fused silica capillary column (HR-SS-10, i.d. 0.25 mm × 25 m, Shinwa Chemical Industries, Ltd.). The oven temperature for analysis was fixed at 170°C. The amount of TG is represented as the total micromoles of fatty acids recovered in the fraction per gram of dry weight of insects (μmol / g.d.w.), the values being corrected by comparison of the recovery of the internal standard.

RESULTS

Changes of TG levels and their fatty acid composition in flown and non-flown males

Table 1 shows the fluctuation of the residual TG levels and their fatty acid composition in abdomens after three-day old males were flown by tethered flight for different periods of time. The TG level per g dry weight gradually decreased with longer duration of flight: from 849.7 μmol in 0 h-flown individuals to 144.3 μmol in individuals which were flown for over 18 h, nearly a 700 μmol decline compared with the start of flight. For comparison, changes of TG levels in the abdomens of non-flown males were determined when they were maintained with only water for ten days, as shown in Table 2. The level of 0 day-old males was 856.6 μmol and that of three day-old males was almost same. Then the levels prominently declined, but were still as high as 548.5 μmol even after ten days of starvation (Table 2), which was nearly equal to the level of 12 h flown males (Table 1).

In TG of the abdomens of non-flown males, lauric acid (C 12:0), miristic acid (C 14:0), palmitic acid (C 16:0), palmitoleic acid (C 16:1), stearic acid (C 18:0), oleic acid (C 18:1), linoleic acid (C 18:2) and linolenic acid (C 18:3) were detected as fatty acids (Table 2). When the insects were flown, the proportion of unsaturated fatty acids in TG's tended to decrease as flight duration was longer: from 52.9% in non-flown individuals, to 26.7% in 12 h-flown ones, and to 8.6% in insects flown over 18 h (Table 1). In non-flown males, the percentages of unsaturated fatty acids in TG's did not change greatly, from 50.1% in non-starved individuals to 44.0% in ten-day-starved ones (Table 2).

Lipid storage in ovaries in female

Tables 3 and 4 show the changes of the ratio of saturated and unsaturated fatty acids in TG's and their levels in the abdomen including ovaries, and also in the ovaries themselves, from non-fed and non-flown females. TG levels in the ovary significantly increased from 150.6

Table 2. Comparioson of the percentage of fatty acid composition in TG's and the amount of TG in the abdomens of non-flown *S. litura* males. 1)

Fatty acid (%) / Days after emergence	12 : 0	14 : 0	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	18 : 3	Other	Saturated	Unsaturated	Level of TG (μmol/g.d.w.) 2)
0	1.3	0.8	45.2	6.2	2.3	31.2	11.0	1.7	0.3	49.6	50.1	856.6 ± 187.9
3	0.3	0.8	44.8	9.9	1.2	28.7	12.5	1.8	0.0	47.1	52.9	849.7 ± 185.4
7	0.5	1.2	54.5	7.0	1.4	25.8	8.6	1.0	0.0	57.6	42.4	603.9 ± 119.3
10	1.2	1.3	52.6	8.5	0.9	23.2	11.3	1.0	0.0	56.0	44.0	548.5 ± 150.1

1) Males were separately maintained in tubes without food during the period as indicated.
2) Values are averages with S.D. (n = 5).

μmol at day 0 to 566.0 μmol at day 3 (t-test, $p < 0.05$), while the levels in abdomens (including ovaries) increased, but not significantly, from 614.3 to 670.0 μmol during the three days of ovarian development (t-test, $p > 0.05$). Thus, TG levels in the ovary were 24.5% of that in the abdomen at day 0, but 84.5% at day 3. During these three days, the ratio of unsaturated fatty acids in the abdomen did not change greatly, but the ratio in the ovaries increased from 3.6% to 48.1%.

DISCUSSION

Flight ability and TG in males

We have shown here that three-day old adult males of *S. litura* used in the present experiment consumed one half of the TG after 15 h of tethered flight (Table 1), so it would be reasonable to infer that they can fly more than 20 h, possibly 30 h, judging from the residual levels of TG. In fact, the longest flight duration observed was about 30 h (data not shown). The males showed similar high flight activity by tethered flight during the first several days after emergence (Murata, 2001). Overseas migration of *S. litura* to Japan, possibly from China (more than a 1000 km distance from Japan), with the aid of winds associated with a typhoon (Murata et al., 1998) and a rain front (Murata, 2001), has been postulated by comparing the occurrence patterns of male moths and meteorological analyses. Our present data demonstrated their high capacity for flight is physiologically guaranteed by high storage of TG's as flight fuel, which would make it possible for them to fly for such a long distance. *Ostrinia furnacalis* and *Plutella xylostella* adults, which have been observed on the East China Sea, in addition to *S. litura* (Kiritani, 1984), were able to fly 13 h and 15 h on average, respectively, when they were tested by overnight tethered flight. Physiological analysis during flight has not, however, been done in these long-distance migratory lepidopteran moths (Shirai, 1993, 1998). Thus, the capability of moths for overseas migration may be estimated by tethered flight experiments.

The composition of TG's in *S. litura* adults consisted of eight main fatty acids, and is similar to that of other long-distance migratory species of Lepidoptera, e.g., *Mythimna separata* (Zongshun & Ouyang, 1995) and *Cnaphalocrosis medinalis* (Murata & Tojo, 2001). In the male adults of *S. litura*, the ratio of unsaturated fatty acids in TG's declined during flight to nearly half after 12 h of flight (Table 1). In non-flown males, TG levels did not change during three days of starvation, and the level in ten day-starved males was nearly the same as that in ones flown for 12 h. During ten days of starvation, the proportion of unsaturated fatty acids in the TG's did not change greatly (Tables 1 and 2). These results clearly demonstrate that TG is not wasted during short-term starvation under non-flown conditions, and unsaturated fatty acids are not consumed even under long-term starvation. Furthermore unsaturated fatty acids in TG's are utilized as a flight energy source during long flight. As most insects require linoleic acid and linolenic acid as essential non-synthetic nutrients (Hirano, 1971), near exhaustion of unsaturated fatty acids in TG as seen in males flown over 18 h may mean the end of flight capacity if only unsaturated fatty acids in TG's are used as fuel for long flights. Work is now in progress to determine if saturated fatty acids are utilized for further flight. In long-distance migratory *C. medinalis*, both TG levels and the ratios of unsaturated fatty acids in TG were found to be significantly lower in adults caught during the migratory season in contrast to those in the second or third non-migratory generation (Murata & Tojo, 2001). We expect to be able to judge the flight experience of males by checking their

Table 3. Comparison of ratios of saturated and unsaturated fatty acids (FA) in TG's and the amount of TG in the abdomens (including ovaries) of non-fed *S. litura* females.

Age (days) after emergence	Saturated FA (%)	Unsaturated FA (%)	Other components (%)	Level of TG (μmol/g.d.w.) 1)
0	61.4	36.8	1.8	614.3 ± 108.4
3	55.8	43.8	0.4	670.0 ± 285.1

1) Values are averages with S.D. (n = 5).

Table 4. Comparison of ratios of saturated and unsaturated fatty acids (FA) in TG's and the amount of TG in the ovaries of unfed *S. litura* females.

Age (days) after emergence	Saturated FA (%)	Unsaturated FA (%)	Other components (%)	Level of TG (μmol/g.d.w) 1)
0	93.7	3.6	2.7	150.6 ± 78.8
3	51.9	48.1	0.0	566.0 ± 47.0

1) Values are averages with S.D. (n = 5).

residual levels of TG's and the fatty acid composition in these TG's.

Lipid storage and reproduction in females

For three days after emergence, when ovaries developed to the mature stage, the amount of TG in the ovaries increased 3.5 fold, approximately 85% of the abdominal TG. During this period the ratio of unsaturated fatty acids in ovarian TG increased to 48% (Tables 3 and 4). As the TG level in abdomen did not significantly change during this ovarian development, it could be assumed that the fatty acids in abdominal TG of newly emerged females were transported to the ovaries, which would occur more intensively for unsaturated fatty acids.

Linoleic acid and linolenic acid are the precursors of prostaglandin which is known to be involved in the regulation of ovarian development and ovipositional behavior (Blomquist et al., 1991). As both fatty acids were predominant components of unsaturated fatty acids in ovarian TG, linoleic acid and linolenic acid seemed to be conveyed from the abdomen, possibly from the fat body, to the ovaries to carry out the functions described above. Some three-day old females with mature eggs could fly more than 12 h by tethered flight and oviposit a similar number of eggs to non-flown individuals, even after such a long flight (Murata, 2001). Thus, we realize that females, even in the fully mature reproductive stage, can be involved in long-distance migration. In mature females, major sources of flight fuels are expected to be the unsaturated fatty acids stocked in the ovaries in the form of TG, if they are obliged to undertake long-distance migration. We are now examining whether unsaturated fatty acids are the sole fuels for long flight in both sexes, whether all females develop ovaries within a short period after emergence, and whether there exist other populations more adapted for migratory life, by exhibiting trade-off of storage lipid for reproduction and migration and different behavioral and physiological traits. Similar studies are desired for other long-distance migratory insects.

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REFFERENCES

BEENAKERS A.M.T., VAN DER HORST D.J. & VAN MARREWIJK W.J.A. 1985: Insect lipids and lipoproteins, and their role in physiological processes. *Progr. Lipid Res.* **24**: 19–67.

BLACKLOCK B.J. & RYAN R.O. 1994: Hemolymph lipid transport. *Insect Bioch. Mol. Biol.* **24**: 855–873.

BLOMQUIST G.J., BORGESON C.E. & VUNDLA M. 1991: Polyunsaturated fatty acids and eicosanoids in insects. *Insect Biochem.* **21**: 99–106.

FOLCH J., LEES M. & STANLEY G.H.S. 1957: A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **266**: 497–509.

HIRANO C. 1971: *Insects and Their Host Plants*. Kyoritsu Press, Tokyo, 202 pp. [in Japanese]

KIRITANI K. 1984: Colonizing insects? What prevented the brown planthopper from establishing itself in Japan? *The Insectarium* **21**: 136–143. [in Japanese]

MIYASHITA K. 1971: Effects of constant and alternating temperatures on the development of Spodoptera litura F. (Lepidoptera: Noctuidae). *Appl. Entomol. Zool.* **6**: 105–111.

MURATA M. 2001: *Researches on Long-Distance Migration in the Common Cutworm, Spodoptera litura*. Ph.D. Dissertation, United Graduate School of Agriculture, Kagoshima University. [in Japanese]

MURATA M., ETOH T., ITOYAMA K. & TOJO S. 1998: Sudden occurrence of the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae) in southern Japan during typhoon season. *Appl. Entomol. Zool.* **28**: 291–301.

MURATA M. & TOJO S. 2001: Seasonal fluctuation of residual flight energy source in the rice leaf roller, Cnaphalocroris medinalis (Lepidoptera: Pyralidae) adults. *Jpn. J. Entomol.* **4**: 1–9. [in Japanese, English abstr.]

NODA T. & KAMANO S. 1988: Flight capacity of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) determined with a computer-assisted flight mill: Effect of age and sex of the moth. *Jpn. J. Appl. Entomol. Zool.* **32**: 227–229. [in Japanese, English abstr.]

OKAMOTO D. & OKADA T. 1968: Studies on the tobacco cutworm, Prodenia litura Fabricius, as an insect pest of the forage crops. *Bull. Cyugoku Natl. Agric. Exp. Stn.* E**2**: 111–144. [in Japanese, English abstr.]

SAITO O. 2000: Flight activity of three Spodoptera litura spp., Spodoptera litura, S. exigua and S. depravata, measured by flight actograph. *Physiol. Entomol.* **25**: 112–119.

SHIRAI Y. 1993: Factors influencing flight ability of male adults of the diamondback moth, Plutella xylostella, with special reference to temperature conditions during the larval stage. *Appl. Entomol. Zool.* **28**: 291–301.

SHIRAI Y. 1998: Laboratory evaluation of flight ability of the Oriental corn borer, Ostrinia furnacalis (Lepidoptera: Pyralidae). *Bull. Entomol. Res.* **88**: 327–333.

WADA S. & SUGANO M. 1972: The practical methods for utilization of thin-layer chromatogaphy to the analysis of glycerolipids from animal tissues. *Sci. Bull. Fac. Agric. Kyushu Univ.* **26**: 505–516.

ZONGSHUN W. & OUYANG Y. 1995: Flight activity and fatty acid utilization in Mythimna separata (Walker) moths. *Entomol. Sinica* **2**: 370–376.

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