

Triacylglycerol storage in the adults of two selected strains of the brown planthopper, *Nilaparvata lugens*

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Abstract

In the brown planthopper, *Nilaparvata lugens*, triacylglycerol (TG) content and its fatty acid composition were analysed by GLC and compared during three days after the final moult. This analysis compared long and short-winged morphs, which were derived from predominantly macropterous and brachypterous strains, and allowed to feed but not mate. For both sexes, the levels of TG on a dry weight basis in the long-wing form were 1.6 times higher shortly after adult eclosion for both sexes than those in the short-wing form. The ratios of TG content between the two wing-morphs increased to 2.8 times for females and 3.7 times for males two days after eclosion, the stage estimated to be the most adapted to migration. Thus the macropters apparently store considerably more fuels for flight than the brachypters. In female macropters, TG per dry weight decreased slightly during the pre-feeding period but returned to the initial level two to three days later due to feeding, possibly providing energy for migration. In contrast, TG level per dry weight in brachypters continuously declined, maintaining TG content per individual with gradual weight gain during this period, suggesting the storage of materials other than TG by feeding for ovarian development. In males, TG level declined more in brachypters than in macropters, possibly reflecting greater adaptation for copulation in the former and migration in the latter. Fatty acid compositions of TG were essentially the same for both morphs and also both sexes at the time of adult emergence, but the composition in brachypterous males changed greatly after one to two days of adult life.

Key words: *Nilaparvata lugens*, wing dimorphism, flight fuel, lipid, triacylglycerol

INTRODUCTION

Lipids are one of the main flight fuels in insects, being stored in the fat body in the form of triacylglycerol (TG) (Beenackers et al., 1985). It is therefore generally expected that long-fliers will store greater quantities of lipids than short-fliers. Many migratory insect species are known to exhibit polymorphism, such as phase polymorphism in some locusts and moths (Uvarov, 1966; Baker, 1981). The adults in phase gregaria are expected to be involved in migration and those in phase solitaria in a more sedentary mode of life, and higher levels of lipid have been reported in gregarious *Locusta migratoria migratorioides* (Ayali and Pener, 1995; Ayali et al., 1996), *Schistocerca gregaria*

(Schneider and Dorn, 1994) and *Spodoptera exempta* (Gunn and Gatehouse, 1987). The morphological and physiological differences between the two phases are not completely distinct, and some intermediate types are possible. Even those in phase solitaria can disperse and migrate in appropriate conditions (Uvarov, 1966), and this makes it difficult to correlate lipid levels with migratory capability. Compared to these insects, migration or dispersal of wing-dimorphic species of aphids, leafhoppers, planthoppers, crickets and so on is limited to long-winged individuals, whereas short-winged or wing-less ones are restricted to a more sedentary life. Greater amounts of lipids may be stored in long-winged adults than in other morphs, as in *Gryllus firmus* (Zera et al., 1994)

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and *Callosobruchus maculatus* (Nwanze et al., 1976). However, more research on the relationship between flight capability and lipid content with these insects is required. A recent study showed no morph-specific differences in lipid storage in the cricket, *Modicogryllus confirmatus*, in which the larger amounts of lipids in long-winged morph were highly correlated to their heavier body weights (Tanaka and Suzuki, 1998).

The brown planthopper, *Nilaparvata lugens* is well known for its wing-dimorphism, exhibiting long-winged (macropterous) and short-winged (brachypterous) forms and long-distance migration overseas (Kisimoto, 1976). Comparisons of meteorological data and records of adults caught by light traps in Kyushu, Japan have led to the conclusion that these insect emigrate to Kyushu mostly from the southern part of China, traveling nearly 2,000 km in 36–48 h with the help of low-jet streams (Sogawa, 1995). Tethered flight experiments showed that macropters of *N. lugens* utilise lipids as the main fuel during long flights and longer fliers have larger initial lipid stores, supporting flights of over 9 h (Padgham, 1983). Furthermore, TG content of *N. lugens* was found to be higher in macropterous adults than in brachypterous ones (Kim et al., 1973).

Although wing morphs in *N. lugens* are largely influenced by environmental conditions, mainly by density (Kisimoto, 1965; Iwanaga and Tojo, 1986), their wing-form responses to density have been demonstrated to be fundamentally under genetic control (Iwanaga et al., 1985, 1987; Morooka et al., 1988). More than 25 generations of successive selection under high density conditions generated several pure lines exhibiting specific wing form and body colour in broad ranges of densities (Morooka and Tojo, 1992), which have been maintained more than 100 generations in our laboratory.

The present study compared TG contents between two wing forms derived from two pure lines, i.e., a macropterous strain and a brachypterous strain reared under crowded conditions, which would function to avoid the density effect and genetic drift on wing-form expression.

MATERIALS AND METHODS

Insects. The macropterous and brachypterous strains mentioned above were reared in cylindrical acryl-resin cages (i.d. 5.4 cm × height 22 cm) containing about 130 rice seedlings (variety *Reiho*) at high-density (200 insects per cage) under a 16L:8D photoperiodic regime at $25 \pm 1^\circ\text{C}$, as previously described (Morooka and Tojo, 1992). New adults with long wings from the macropterous strain and those with short wings from the brachypterous strain were collected within 6 h after emergence and about 100 males and females were separately enclosed in the new cages with rice seedlings, in order to keep non-mating and highly crowded conditions over 50 per cage which were effective to avoid of genetic drift (Morooka and Tojo, 1992). Twenty individuals of respective sexes were sampled 0, 24, 48 and 72 h after the initial collection, killed in a freezer, and lyophilised (5 batches per sampling time).

Lipid extraction. Lipid extraction was performed according to Folch et al. (1957). Briefly, 20 lyophilised insects were homogenised in 7.5 ml methanol followed by 15 ml chloroform and then incubated at 37°C for 30 min under shaking. After cooling, the homogenate was made up to 25 ml with chloroform–methanol (2 : 1, v/v) and filtered (Whatman No. 2 filter paper). Distilled water (4.7 ml) was added, the mixture was shaken and then placed at 4°C overnight. The chloroform layer was collected and evaporated to dryness under nitrogen.

Lipid separation. Lipid separation was carried out by thin-layer chromatography (TLC) according to Wada and Sugano (1972). The residue obtained in the above-mentioned procedure was dissolved in 2 ml of petroleum ether and $40 \mu\text{l}$ was spotted onto a silica gel G plate (Whatman K5, 20 cm × 20 cm), which had been activated for 2 h at 110°C , and run in a separation solvent, 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 vapour. The spot corresponding to TG was identified by comparing the *R_f* value of standard TG from rat liver, cut from the plate with a fine stainless cutter.

Triacylglycerol analysis. The amount of TG and its fatty acid composition were determined by gas-liquid chromatography (GLC). After addition of an internal standard (pentadecanoic acid, Sigma Chemical Co.) to the TG fraction, the fatty acids in it were converted to their methyl esters by refluxing in oxygen-free methanol-hydrochloric acid (5 : 1, v/v) at 65°C for 3 h. The methyl esters were then extracted twice with 2 ml hexane and concentrated under nitrogen. The methyl esters were subjected to GLC (Shimadzu GC-14B) using a hydrogen flame ionization detector and a capillary column (HR-SS-10, i.d. 0.25 mm × 25 m, Shinwa Chemical Industries, Ltd.). The column temperature for analysis was fixed at 170°C. The amount of TG was represented as the total micromoles of fatty acids recovered in the fraction per dry weight of insects ($\mu\text{mol/g d.w.}$), the values being corrected by comparison of the recovery of the internal standard.

RESULTS

Changes in TG content, determined by GLC analyses were compared on a dry weight basis (Fig. 1a). TG levels (fatty acids, $\mu\text{mol/g d.w.}$) in new adults were 1.6 times higher in macropters (females and males, 549.5 ± 40.3 and 543.7 ± 43.2 , respectively) than in brachypters (345.1 ± 40.5 and 338.8 ± 28.3 , respectively), but did not differ between sexes. The subsequent change in TG levels depended on sex and wing-form. In long-winged adults, the TG level declined slightly within 24 h after eclosion with both sexes, but in females it returned to the original level, 24 to 48 h later, while the TG in males further declined to one third of the original level 72 h after eclosion. In short-winged adults, the TG level did not change within 24 h after eclosion, but it declined prominently 24 h later to two thirds of the original level in the females and one third in the males.

Figure 1b shows that the long-winged adults of *N. lugens* lost dry weight more prominently in females than in males within 24 h after adult eclosion, but the females regained their weight at eclosion within a further 48 h, during which time the weight of males did not change. Short-winged adult females continued to gain dry weight during the first three days of adult

life, while the males kept their weight at a constant level.

Figure 1c shows the changes in TG contents per individual insect for different wing-forms and sexes. In long-winged adults, the TG content showed a prominent decrease during the first 24 h of adult life with both sexes, but in the females it returned to the level of new adults during the following 48 h, while the level in the males declined further. In short-winged adults, the content in the females was essentially the same during the first three days of adult life, but in the males it considerably decreased during the first one and two days of adult life.

In Fig. 1, the effects of one day starvation on these parameters in newly ecdysed adults were also compared between the two wing-morphs. When compared with non-starved individuals, essentially no effects on dry weight and TG contents per dry weight and per individual were caused by starvation of macropters, but the levels of these three characteristics significantly reduced in the starved brachypters.

Table 1 indicates that the fatty acid compositions of TG in newly emerged adults of both sexes and of both wing-forms were nearly same: palmitic acid ($C_{16:0}$) and oleic acid ($C_{18:1}$) occupied 46–48 and 31–35% of the components, respectively. During the first three days of adult life, palmitic acid and oleic acid comprised nearly 80% of the TG fatty acids in long-winged females and males, although the total contents greatly differed between sexes (Fig. 2). However, in short-winged adults, the composition changed dramatically between sexes between 24 and 48 h of adult life, when the relative ratio of oleic acid suddenly declined in males.

DISCUSSION

In this article, using two genetically defined strains of *N. lugens*, which predominantly exhibited one of the two wing-forms, we demonstrated more storage of TG on a dry weight basis in macropterous adults than in brachypterous ones at the time of adult emergence. The results also showed wing-form- and sex-specific changes of TG levels during the early adult stage in non-flying and non-mating conditions. The loss of dry weight and decrease of TG

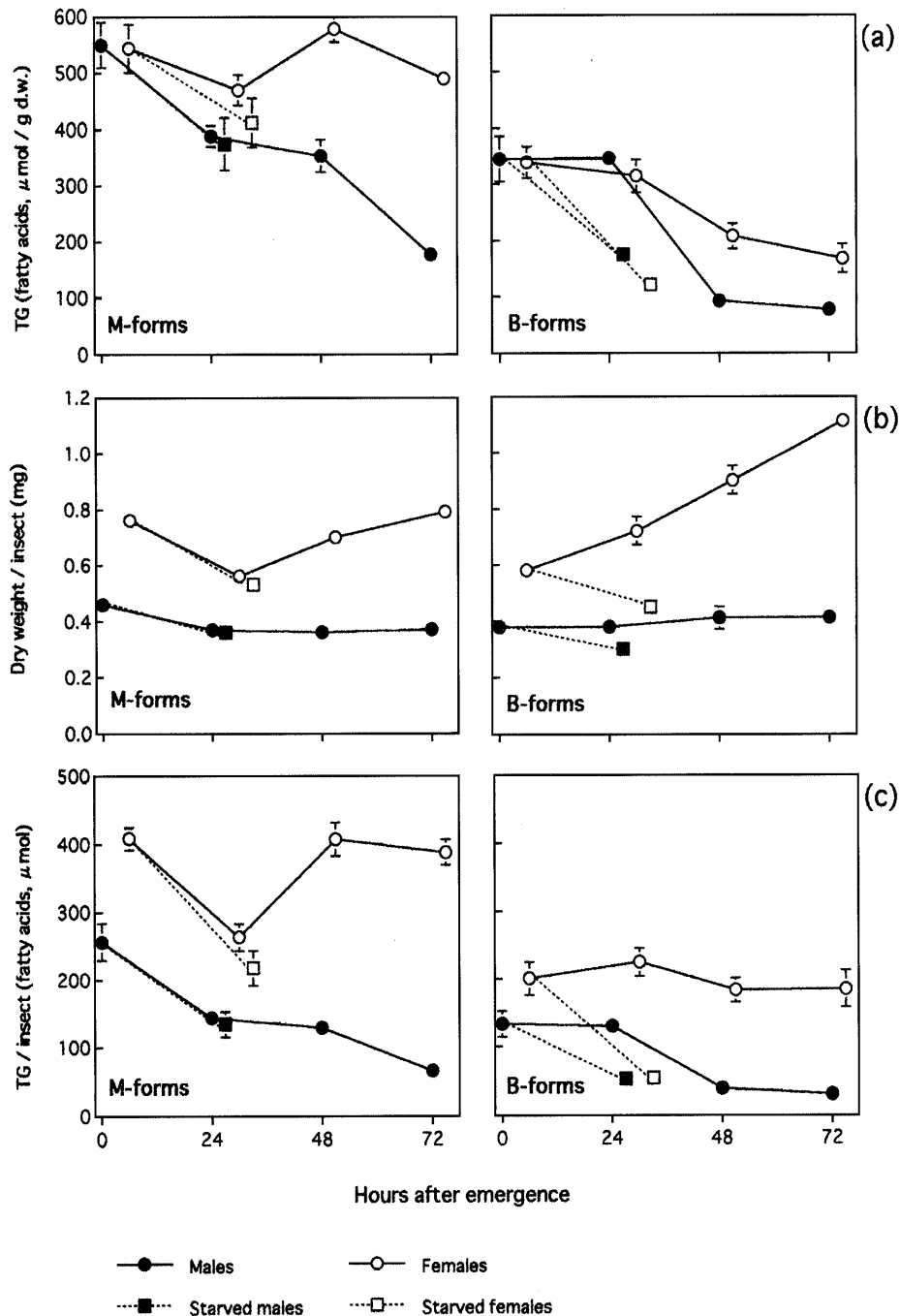


Fig. 1. Comparison of changes in TG contents per g dry weight (a) and per individual (c), and dry weight (b). Macropterous-forms (M-forms) and brachypterous-forms (B-forms) were obtained from predominantly macropterous and brachypterous strains, respectively, reared at 25°C in crowded conditions, their stages synchronised to be within 6 h after emergence. Females (○) and males (●) were separately reared in non-flyable conditions with rice seedlings (solid line). One group was starved for 24 h after adult emergence (dotted line, □, ■). For each analysis, 20 individuals were used. Values are means with S.E. ($n=5$).

content during the first 24 h of adult life in macropterous females and males (Fig. 1) support the presence of a pre-feeding period at this age. This was confirmed by the absence of an effect of starvation on both dry weight and TG content during this period. Feeding after this period caused the macropterous females to gain

weight and accumulate TG to levels comparable with those of new adults, indicating *de novo* TG synthesis, before migration and ovarian development, which starts around 72 h after adult emergence (Ayoade et al., 1996). We also demonstrated a rapid decline in the TG level of macropterous males after two days of adult life.

Table 1. Comparison of fatty acid compositions of TGs in adults within 6 h after emergence between two wing morphs of *N. lugens*

Fatty acids	Males		Females	
	M-forms	B-forms	M-forms	B-forms
C _{12:0}	1.2	0.7	0.6	0.4
C _{14:0}	1.4	2.4	1.3	1.5
C _{16:0}	45.7	48.2	46.3	46.9
C _{16:1}	0.6	1.1	1.0	1.0
C _{18:0}	5.8	7.4	5.1	5.7
C _{18:1}	34.0	31.3	34.2	34.7
C _{18:2}	11.2	8.9	11.4	9.7

Values are ratios of each fatty acid in TGs (%).

At this stage, the males became more active, and most of them left the rice-seedlings and moved to the top of the rearing cage. Other researchers also observed active flying behaviour in macropterous males at this age (Ohkubo, 1973; Padgham, 1983), and the loss of TG in the males may be due to an increased energy expenditure. From these results, we conclude that the macropterous adults are energetically adapted for migration 48 h after adult emergence, with a high TG level, but in a reproductively immature condition.

The changes of TG in brachypterous adults (which cannot fly and reproductively mature earlier than macropterous adults) considerably differed from those in macropterous ones. We can conclude that there was no or a very-short pre-feeding period in the brachypters, as one day starvation after adult emergence caused a significant reduction in dry weight and TG levels per dry weight and per individual, compared to non-starved ones (Fig. 1). In the females, although TG content per individual was kept at a constant level during the first three days of adult life, the TG content continuously declined, because of weight gain throughout this period. This suggests active feeding and accumulation of substances other than TG, possibly for yolk deposition, which initiates within 48 h of adult life (Ayoade et al., 1996). In the males, the decline of TG per dry weight between 24 and 48 h of adult life seems to be caused by their increased reproductive activity.

The present results indicate that physiological events during the nymphal period are spe-

cifically related to wing-form, as reflected by a 1.6 times higher level of TG on a dry weight basis in macropters than in brachypters at adult emergence. Forty-eight hour-old adults are highly suited to migration, as suggested from the results in Fig. 1 and also by Padgham (1983). We can therefore conclude that macropterous females and males before migration have 2.8 and 3.7 times more TG on a dry weight basis, respectively, compared to the same-aged brachypters. A previous study with new adults of *N. lugens* reported essentially similar differences between the two wing forms: the ratio of TG content on a dry weight basis in macropters to that of brachypters was 1.7 in female and 1.4 in males (Kim et al., 1973). Several differences between these two studies make a direct comparison of results difficult. In the previous study, the adults were collected within 24 h after eclosion, but the present sampling was done within 6 h of eclosion. As the level of TG considerably changed with adult age (Fig. 1), care should be taken to synchronise the development of insects within a few hours. As the insects in the study by Kim et al. were field-collected, development of the wing-forms might have been very senile to rearing conditions, and results may not directly reflect morph-specific development. The present approach with two wing-morphs derived from two genetically defined strains reared under crowded conditions should minimize the influences of environmental differences and genetic drift (Morooka and Tojo, 1992). As in fields, there are populations showing various genetic variation in the wing-form responses to nymphal density (Iwanaga et al., 1985, 1987; Morooka et al., 1988), it is desirable to check if the differences in TG storage found between two wing-forms deprived from genetically pure lines are also relevant to those in the field populations.

The present study demonstrated that the fatty acid composition of TG during the early stage of adult life in *N. lugens* (Fig. 2), was essentially the same for both wing-forms and sexes, with palmitic acid (C_{16:0}) and oleic acid (C_{18:1}) occupying 46–48 and 31–35% of the components, except for the brachypterous males, in which the ratio of oleic acid prominently decreased after 24 h of emergence. Previous work

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