

# Continuous Variation in Wing Length and Flight Musculature in a Tropical Field Cricket, *Teleogryllus derelictus*: Implications for the Evolution of Wing Dimorphism

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**Abstract.** Wing polymorphism with continuous variation in flight musculature was discovered in a tropical field cricket, *Teleogryllus derelictus* Gorochov derived from Java, Indonesia. In this species, typical short-winged (SW) and long-winged (LW) morphs often observed in other species of cricket were found, but individuals with intermediate wing lengths (IM morph) were also observed. However, the frequency distribution in relative wing lengths, *i.e.* hind wing length (HW)/fore wing length (FW), was bimodal with a relatively low frequency of IM morphs. Flight muscle mass at adult emergence was directly related to HW/FW and the variation was continuous. It changed little after adult emergence in SW morphs, but showed a >70% increase in some LW morphs whereas a reduction in mass due to histolysis occurred in IM and other LW individuals. Flight muscle growth and histolysis were accompanied by conspicuous color changes apparently related to cytochrome *c*. The total fat and triacylglycerol contents of the body were closely related to body weight, but not to HW/FW. Some LW adults flew out of the rearing tanks within a week after adult emergence. In response to a synthetic adipokinetic hormone (AKH), both LW and SW adults were capable of forming low-density lipophorin (LDLp), which is known to be important in mobilizing lipid fuel for flight activity in other insects. The methanol extracts of corpora cardiaca taken from both LW and SW morphs showed AKH activity, indicating that inter-morph physiological differentiation has not occurred in the lipid-mobilization system. The present results with *T. derelictus* do not support Roff's hypothesis that a reduction in flight muscles occurred before that in wing length during the course of evolution for wing dimorphism, but may suggest an alternative evolutionary pathway.

**Key words:** Wing dimorphism, evolution, flight muscle, flight activity, lipophorin, adipokinetic hormone, *Teleogryllus derelictus*.

## Introduction

Wing dimorphism is widespread among many orders of insects, and has been studied intensively from various points of view (Alexander, 1961; Southwood, 1962; Johnson, 1969; Dingle, 1975; 1985; Harrison, 1980; Pener, 1985; Roff, 1986; Fujisaki, 1994; Zera & Denno, 1997). In general, there are two morphologically distinct morphs which have long and short wings, respectively. The long-winged (LW) morph is normally able to fly and sometimes called the macropterous morph. On the other hand, the short-

winged (SW) morph cannot fly and is often referred to as the micropterous or brachypterous morph. In some species such as aphids, the flightless morph lacks wings and is called the apterous morph. Besides flight capability, the two wing morphs often display various behavioral and physiological differences. For example, short-winged male adults are more aggressive or more vigorously call females than long-winged counterparts in some species (Crespi, 1986, 1988; Crnokrak & Roff, 1998). It is common that reproduction begins earlier in short-winged females than in long-winged ones (Dingle, 1985; Roff, 1986). In a few cases, earlier mating or earlier development of the reproductive accessory organ in males has been demonstrated

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(Fujisaki, 1992; Tanaka, 1999). These morph-specific differences are assumed to be related to fitness, and often regarded as factors closely associated with the evolution of wing dimorphism.

Roff (1986) proposed a possible evolutionary sequence of wing dimorphism in insects. In this sequence, (1) behavioral differentiation occurs first in a monomorphic macropterous population and selection favors an increasing proportion of nondispersers as habitat stability increases. In the next step (2), individuals that produce wings but not flight muscles appear in the population. Then, (3) there is a reduction of wings and other body parts concerned with flight. According to this hypothesis, inhibition of flight muscle development occurs before that of wing development. Thus, he described "it is not unexpected to find some species in which all individuals are fully winged but which are polymorphic with respect of wing-muscle development". Some coleopterans such as *Anomala schonfeldti* (Tada *et al.*, 1993) and *Heptophylla picea* (Tada *et al.*, 1995) may be good examples supporting his statement, but to our knowledge, no example has been reported against this hypothesis with respect to the evolution of flightless forms. In this article, we will provide an example that may indicate an alternative pathway leading to the evolution of wing dimorphism.

During the course of an excursion to Indonesia in 1996, we collected several species of crickets. One of the species was *Teleogryllus derelictus* Gorochov, which is distributed widely in South-east Asia (Gorochov, 1985). Soon after we started rearing them in the laboratory, we noticed that this species had individuals with long or short wings, as commonly observed in other wing-dimorphic insects. However, the variation in wing length was not discrete, but appeared to be continuous. Furthermore, individuals with intermediate wing lengths were found to have medium-sized flight musculature at adult emergence. In the present article, we describe the morphological, behavioral and physiological traits related to dispersal dimorphism in *T. derelictus* and provide evidence to indicate that a reduction of wing length may occur before or at the same time as that of flight muscles in crickets.

Flight activity is sustained with various fuels such as lipids, carbohydrates and amino acids depending on the species (Beenackers *et al.*, 1985). In tethered crickets, there is a switch-over of fuel from carbohydrate to lipid during flight (Tanaka *et al.*, 1999b), as observed in locusts (Beenackers *et al.*, 1985; Chino, 1985; Goldsworthy, 1983, 1990; Pener *et al.*, 1997). The mobilization of lipid into the hemolymph is stimu-

lated by the release of the adipokinetic hormone (AKH) from the corpora cardiaca (CC) in crickets (Gäde & Rinehart, 1987; Woodring *et al.*, 1990; Cusinato *et al.*, 1991; Tanaka *et al.*, 1999b). Lipophorin, the major insect hemolymph lipoprotein, receives diacylglycerol (DG) from the fat body stores of triacylglycerol (TG) and carries it to various organs such as flight muscles (Chino, 1981, 1985; Blacklock *et al.*, 1995). In a LW cricket, *Gryllus bimaculatus*, lipid loaded lipophorin (low-density lipophorin, LDLp) is formed in response to AKH or flight activity (Tanaka *et al.*, 1999b). In the present study, adults of *T. derelictus* were injected with a synthetic AKH and CC extracts from LW and SW adults to determine if any differentiation had occurred between the flight-capable and flightless morphs in terms of the ability to form LDLp and the possession of an AKH. These results will be discussed in relation to the evolution of wing dimorphism.

## Materials and Methods

### *Insects*

Crickets used in this study came from Subang (altitude < 80 m; latitude 6° 35' S), Province of West Java, Indonesia. The annual average precipitation is about 2,625 mm, but little rain falls during the period from June to September (< 100 mm). Having been air-transported to Japan, they were reared in a large plastic container at 30°C in a photoperiod of 16-h light and 8-h dark. They fed on dry feed (Insect Pellet, Oriental Yeast Co., Tokyo) and carrot. Water was supplied in vials plugged with cotton, and eggs were deposited into the cotton. Groups of 50 hatchlings were reared in small plastic containers (diameter, 15 cm; height, 9 cm) until reaching a mid nymphal instar according to the rearing method of Tanaka (1993) for *Modicogryllus confirmatus*, and then transferred to large plastic tanks (32 × 48 × 30 cm) with a lid covered with wire-mesh.

### *Measurements of body dimensions*

Head width was measured with an ocular micrometer installed in a stereomicroscope. Wings were removed from specimens and the right fore wing length (FW) and hind wing length (HW) were measured to the nearest 0.1 mm with a pair of calipers.

### *Mass determinations of whole body, flight muscles, and reproductive organs*

All mass determinations were made to the nearest 0.01 mg with an electronic balance (Mettler AT 201)

both before and after drying each individual or organ. Day-0 adults used were 8–24 hours posteclosion. Some individuals were given dry feed and water for 7 days after adult emergence to determine if flight muscle growth would occur or not. The dorsolongitudinal muscle (DLM) and dorsoventral muscle (DVM) were separately removed from each specimen and dried at 100°C over night in an oven by the method of Tanaka (1993).

#### Absorption spectra

The absorbance spectra of the flight muscle were measured from 300 to 700 nm with Spectra MAX 250 (Molecular Devices, Japan). DLM removed from each specimen was homogenized in extraction buffer (0.1 M Tris-HCl, pH 8.3, 0.8 M NaCl, 2 mM phenylmethylsulfonyl fluoride, 0.2% sodium deoxycholate, 0.2% Triton X) with an ultrasonic homogenizer (Ultrasonic Generator Model US-150) for about 5 seconds. The homogenate was centrifuged at about 10,000g for 15 min. The supernatant was used to observe the absorbance spectra.

#### Determination of fat and triacylglycerol content

Each specimen was freeze-dried, weighed and extracted with 2 ml of a chloroform/methanol (2 : 1 = v/v) solution in a tube (6 ml vol., PP sample tube, Maruemu Co., Japan) for 24 hours. Extraction was repeated with another 2 ml of the solution, and the difference between dry weight and lean dry weight was regarded as total fat content. The solutions used for extraction of each cricket were pooled and TG content was quantified by a TLC/FID analyzer (Iatroscan, MK 25; IATRON Lab. Co.) according to the method of Tanaka *et al.* (1999a). Cholesterol acetate (Wako, Japan) was used as an internal standard (1 mg). The standard line was drawn using various amounts of tripalmitin (0–25 mg; Wako, Japan), and TG content in each pooled sample was determined by the following equation:  $\log Y = 0.7263 \log X + 0.0514$ , where  $Y$  is TG (mg) and  $X$  the area ratio of the TG peak to the internal standard peak ( $r = 0.999$ ;  $df = 7$ ;  $P < 0.001$ ).

#### Flight activity

To determine when crickets start flight activity, 4–7 crickets were held in plastic tanks (32 × 48 × 30 cm) and the number of crickets that flew out of the tanks was recorded for a week. This method has been used for other crickets by Walker (1987) and Tanaka *et al.* (1999b). In a preliminary test, all SW morphs tested remained in the tanks during the observation period (N = 11), whereas some LW morphs left the tanks (N

= 10). In this study, a total of 35 LW adults were tested for each sex, and we assumed that those which left the tanks had taken off for flight according to Tanaka *et al.* (1999b). The crickets that were found outside the tanks were collected every day and some dissected to observe the status of the flight muscles.

#### Effects of adipokinetic hormone (AKH) and extracts of corpora cardiaca (CC) on lipophorin

Crickets were injected with 10 pmol AKH (Grb-AKH) in 10  $\mu$ l of insect Ringer's solution and held at 30°C for 90 min. Grb-AKH has been isolated from *Gryllus bimaculatus* and *Acheta domesticus* (Gäde & Rinehart, 1987; Woodring *et al.*, 1990; Cusinato *et al.*, 1991), and the hormone used in the present study was synthesized by Accord Co. (Japan). CC removed from LW and SW adults were extracted with 80% methanol. The supernatant was dried, mixed with water and used for injection. The methods to collect hemolymph samples and to visualize lipophorin bands have already been described (Hiraoka & Katagiri, 1992; Tanaka *et al.*, 1999b).

## Results

#### Wing lengths

Fig. 1 shows photographs of female adult specimens with various wing lengths. As reported for other families of cricket (Masaki & Oyama, 1963; Alexander, 1968; Walker & Sivinski, 1986; Shimizu & Masaki, 1993), the hind wings of *T. derelictus* extended far beyond the tip of the cerci in some individuals (Fig. 1A), whereas they were completely concealed under the fore wings in other individuals (Fig. 1E). Between such extreme cases, individuals with intermediate hind wing lengths were also observed (Fig. 1B, C and D). Basically the same variation was found in males, as will be described below. Fig. 2 illustrates the morphology of hind wings of various sizes. The shape was similar, but not exactly homologous between large and small wings. Wing venation was less obvious in a small wing than in a large one, particularly the jugum and vannus regions.

According to the standard method used for comparisons of *Gryllus* species by Walker & Sivinski (1986), HW/FW ratios were adopted to determine the frequency distribution of various wing lengths (Fig. 3). In both sexes, a bimodal pattern was obtained, indicating the existence of two major groups representing relatively long and short wings, respectively. However, the variation was continuous with intermediate forms. Females tended to show larger HW/FW ratios

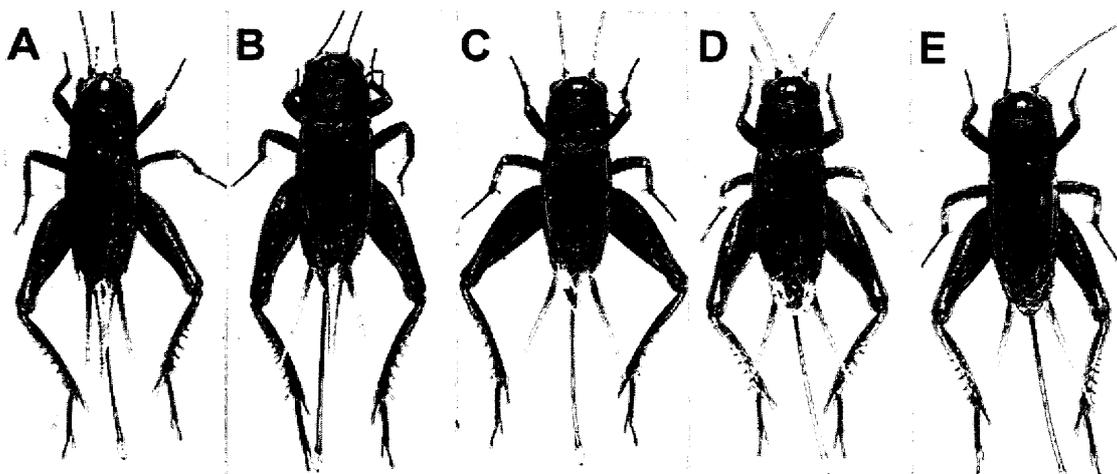


Fig. 1. Photographs showing variation of wing length in female adults of *T. derelictus*. Hind wings are long in (A) and short in (E), whereas those in (B), (C) and (D) are intermediate in length.

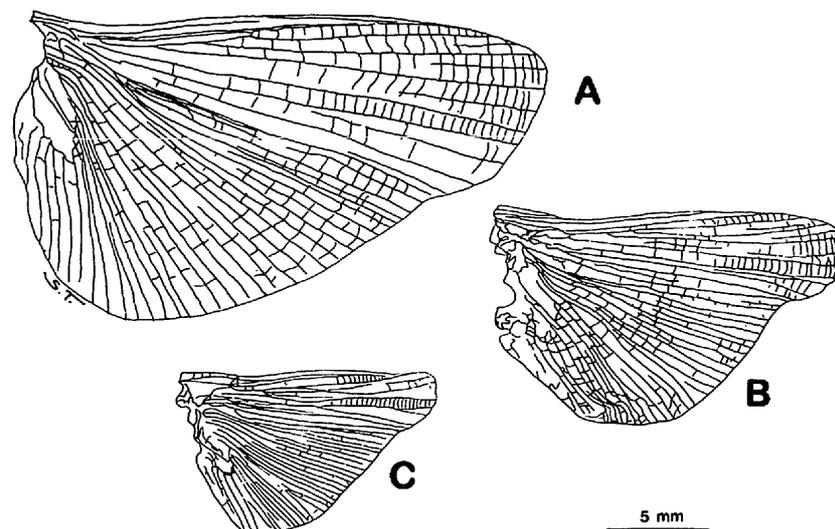


Fig. 2. Drawings of hind wings of *T. derelictus*. Hind wing length/fore wing length was 1.54, 1.01 and 0.79 in (A), (B) and (C), respectively.

than males. In this study, individuals with a HW/FW ratio less than 1.10 were arbitrarily defined as short-winged (SW), those with a ratio larger than 1.35 as long-winged (LW), and those in between (1.10–1.35) as intermediate (IM) morphs.

FW and HW are plotted against head width in Fig. 4. In both sexes, variation in FW was continuous and positively correlated to head width ( $r=0.280$ ,  $N=134$ ,  $P<0.05$  in females;  $r=0.393$ ,  $N=131$ ,  $P<0.001$  in males; Figs. 4A & B). However, the correlation coefficients were rather small, and the range of variation in FW was conspicuously smaller in males (variance=0.482;  $N=131$ ) than in females (variance=1.126;  $N=134$ ). These results indicated that in general wing lengths were positively correlated with body size, but FW varied less in males than in females. The latter fact may be related to the stridulating behavior

of males. On the other hand, HW varied greatly in both sexes, but showed no direct correlation with head width. FW and HW were positively correlated to each other in both females ( $r=0.834$ ,  $N=133$ ,  $P<0.001$ ) and males ( $r=0.668$ ,  $N=131$ ,  $P<0.001$ ), and significant positive relationships were also obtained when SW, IM and LW morphs were calculated separately (Figs. 4C & D).

#### Flight muscles

The DLM and DVM were separately removed from each adult to determine the dry weight (Tables 1 & 2). Their relationships to HW/FW are presented only for females in Figs. 5A and B, because both sexes showed similar patterns. Both DLM and DVM masses at adult emergence had a positive correlation with HW/FW, and like wing lengths the variation was con-

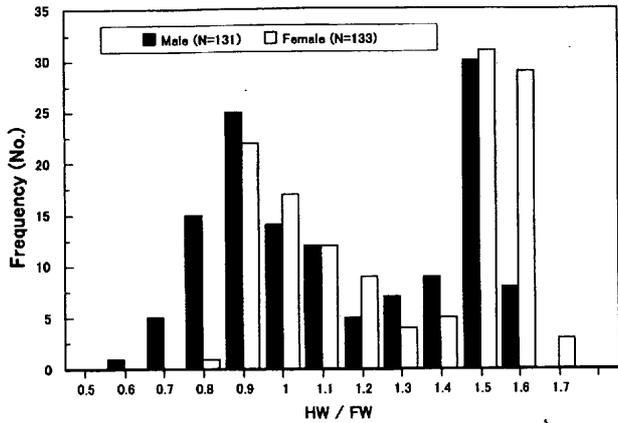


Fig. 3. Frequency distribution of various HW/FW ratios in *T. derelictus*. HW/FW means hind wing length/fore wing length. Individuals with a HW/FW ratio less than 1.10 were defined as short-winged (SW), those with a ratio larger than 1.35 as long-winged (LW), and those in between (1.10-1.35) as intermediate (IM) morphs.

tinuous. A different picture was shown in 7-day-old adults. In SW morphs, no significant change was detected in mean DLM mass during the 7-day period. However, this muscle was apparently atrophied in IM and some LW morphs, whereas it grew heavier in other LW morphs (Fig. 5A). In the latter case, flight muscle growth was observed only in those individuals with a HW/FW ratio larger than 1.35. DVM mass changed little after adult emergence in SW and IM morphs, but in LW morphs, it decreased in some individuals and increased in others (Table 1 & Fig. 5 B). The increase in mass during the first week in LW females after excluding histolyzed individuals amounted to 97.1% for DLM and 71.7% for DVM, with an average of 74.3% for the two muscles combined (Table 1). The corresponding values for LW males were slightly larger, though the sample size for this sex was small. A close association was found between DLM and DVM masses at adult emergence (Table 2

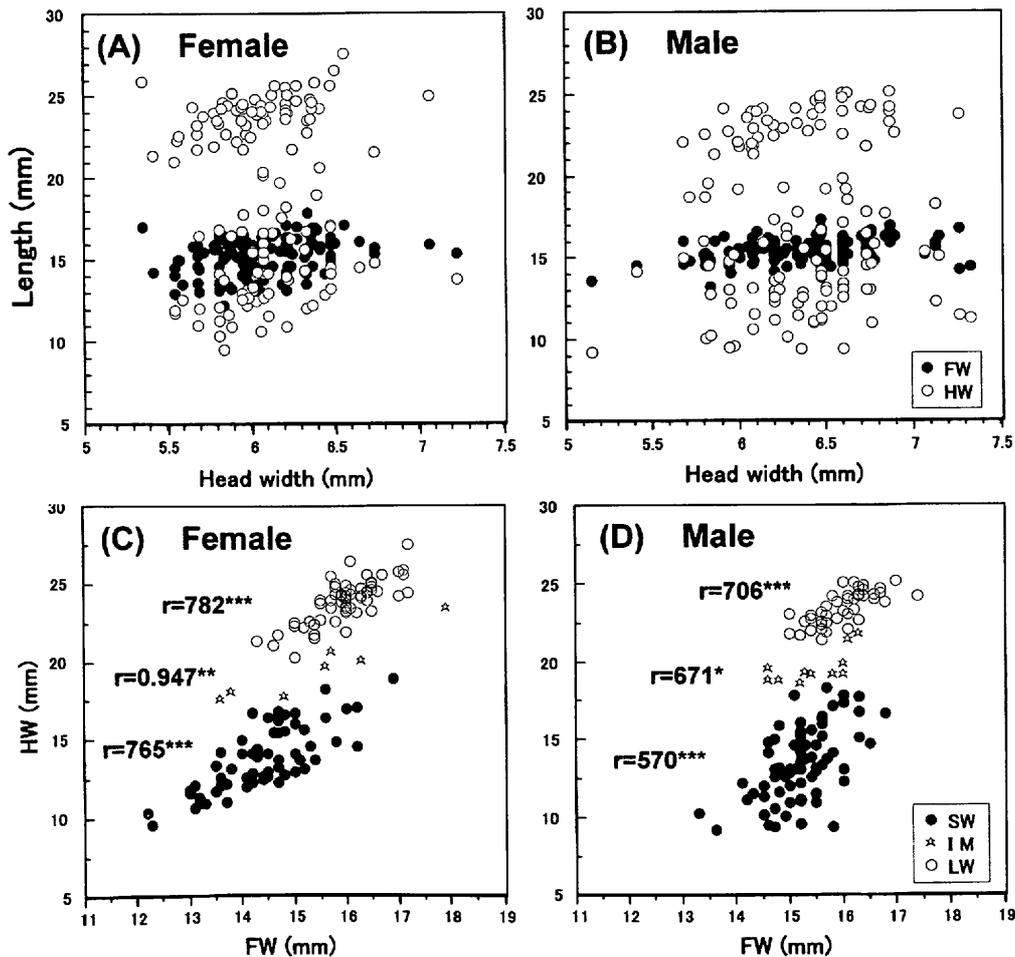


Fig. 4. Fore wing length (FW) and hind wing length (HW) plotted against head width (A & B) and relationships between FW and HW in different wing morphs of *T. derelictus* (C & D). The value of  $r$  was significant at 5% (\*), 1% (\*\*), or 0.1% (\*\*\*) level.

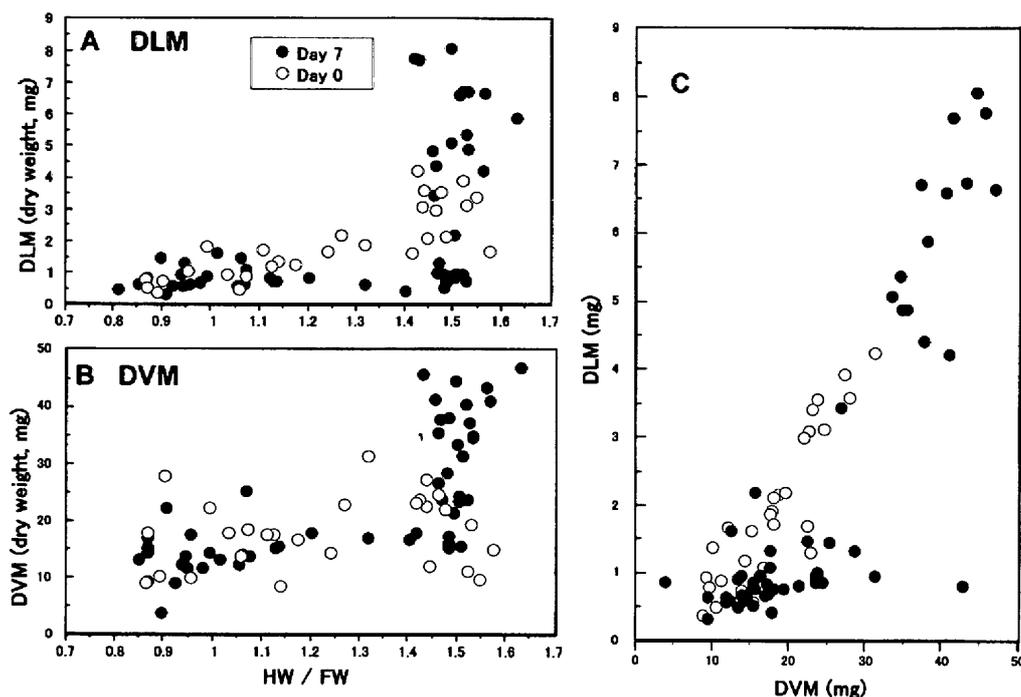


Fig. 5. Flight muscle growth and histolysis during the first 7 days of adulthood in *T. derelictus*. A, DLM (dorsolongitudinal muscle) mass vs. HW/FW; B, DVM (dorsoventral muscle) mass vs. HW/FW; C, DLM mass vs. DVM mass.

Table 1. Dry weight (mean  $\pm$  SD, mg) of flight muscles in short-winged (SW), intermediate (IM) and long-winged (LW) morphs of *T. derelictus* on days 0 and 7 of adulthood.

Morph	Sex	DLM <sup>a</sup>		DVM <sup>b</sup>	
		day 0	day 7	day 0	day 7
SW	♀	0.87 $\pm$ 0.41 (10)	0.86 $\pm$ 0.35 (20)	12.53 $\pm$ 3.23 (10)	14.21 $\pm$ 4.44 (20)
	♂	1.12 $\pm$ 0.72 (18)	1.06 $\pm$ 0.34 (23)	11.84 $\pm$ 3.27 (18)	14.84 $\pm$ 3.44 (23)
IM	♀	1.63 $\pm$ 0.33 (7)	0.79 $\pm$ 0.07 (5)*	16.42 $\pm$ 4.16 (7)	16.60 $\pm$ 0.97 (5)
	♂	1.50 $\pm$ 0.83 (3)	0.77 $\pm$ 0.27 (6)*	12.99 $\pm$ 1.45 (3)	14.68 $\pm$ 4.16 (6)
LW	♀	3.09 $\pm$ 0.78 (12)	3.52 $\pm$ 2.68 (29)	23.16 $\pm$ 4.50 (12)	30.49 $\pm$ 9.94 (29)
			1.21 $\pm$ 0.76 <sup>c</sup> (15)*		21.92 $\pm$ 4.90 <sup>c</sup> (15)*
	♂	2.53 $\pm$ 0.53 (5)	6.09 $\pm$ 1.24 <sup>d</sup> (14)*	18.83 $\pm$ 4.36 (5)	39.67 $\pm$ 4.23 <sup>d</sup> (14)*
			2.54 $\pm$ 2.07 (23)		24.70 $\pm$ 8.30 (23)
			1.26 $\pm$ 0.35 <sup>c</sup> (16)*		20.17 $\pm$ 3.59 <sup>c</sup> (16)*
		5.47 $\pm$ 1.02 <sup>d</sup> (7)*		35.82 $\pm$ 6.22 <sup>d</sup> (7)*	

<sup>a</sup> Dorsolongitudinal muscle.

<sup>b</sup> Dorsoventral muscle.

<sup>c</sup> Individuals with whitish muscles.

<sup>d</sup> Individuals with dark pink muscles.

\* Significantly different from the mean on day 0 ( $P < 0.05$ ; Games-Howell test).

& Fig. 5C). On day 7, DLM was either large or small, whereas DVM was rather continuous in mass. Large DVM was closely associated with large DLM, though the association was not perfect.

Another variation in flight muscles was found in color (Figs. 6 & 7). The differences in color were likely to be due to the differences in amounts of cytochrome *c*, because the darkness of the color was related to the relative absorbance peak similar to that

of cytochrome *c* (415 nm). At adult emergence, the color of the muscles was pinkish in LW morphs (Fig. 6A), whereas it was whitish or yellowish in SW morphs (Fig. 6C). The flight muscles in IM morphs were intermediate in color (Fig. 6B). The darker the color the higher the absorbance peak at about 410 nm. The well developed flight muscles in 7-day-old LW morphs assumed dark pink with the highest absorbance peak at about 410 nm (Figs. 6D & 7B) and the

Table 2. Flight muscle masses (dry weight, mg) of *T. derelictus* at adult emergence.

Variables	Sex	Regression	$R^2$	$R$	N	$F$	$P$
DLM <sup>a</sup> vs. HW/FW <sup>c</sup>	♀	$Y=3.87X-2.84$	0.667	0.816	28	51.99	<0.0001
	♂	$Y=2.35X-1.00$	0.465	0.682	26	20.85	<0.001
DVM <sup>b</sup> vs. HW/FW	♀	$Y=19.12X-5.70$	0.514	0.736	28	30.64	<0.0001
	♂	$Y=12.07X+0.81$	0.505	0.711	26	24.49	<0.0001
DLM vs. DVM	♀	$Y=0.16X-0.95$	0.781	0.884	28	92.87	<0.0001
	♂	$Y=0.17X-0.89$	0.740	0.860	26	68.43	<0.0001

<sup>a</sup> Dorsolongitudinal muscle.

<sup>b</sup> Dorsoventral muscle.

<sup>c</sup> Hind wing length/Fore wing length.

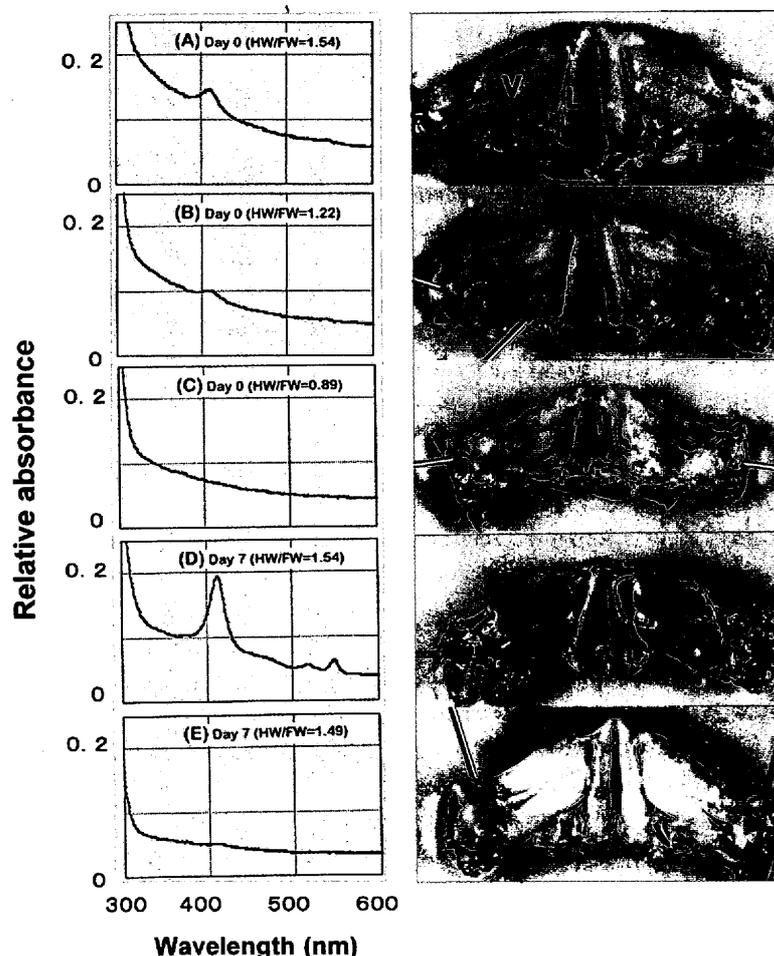


Fig. 6. Absorption spectra and photographs of flight muscles in different wing morphs of *T. derelictus*. A, 0-day-old LW morph; B, 0-day-old IM morph; C, 0-day-old SW morph; D & E, 7-day-old LW morphs. L, DLM; V, DVM.

histolyzed muscles in other LW morphs lost the pinkish pigment completely (Figs. 6E & 7B).

#### Body weight and lipid content

Measurements of dry body weight at adult emergence indicated that LW morphs were heavier than SW, though the difference was significant only in females (Table 3). A significant but weak correlation was also found between body weight and HW/FW in

both sexes (Fig. 8A). The amount of total fat (mg/insect) was directly correlated to body weight (data not shown), and so was the total fat content (% dry body weight) (Fig. 8B). Total fat content apparently showed no significant correlation to wing length in either sex (Fig. 8C). No significant difference was found in mean total fat content between SW and LW in either sex when analyzed with dry body weight as a covariate ( $P > 0.05$ ; Table 3). SW males had 20.9%

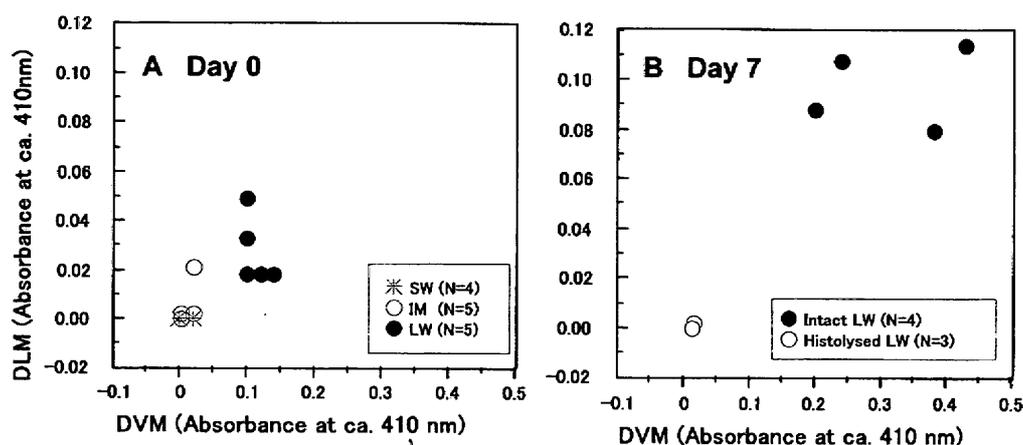


Fig. 7. Relationships between the DLM and DVM in absorbance peak at ca 410 nm in different wing morphs of *T. derelictus* on day 0 (A) and day 7 (B).

Table 3. Dry body weight and lipid content (mean  $\pm$  SD) in short-winged (SW) and long-winged (LW) morphs of *T. derelictus* at adult emergence.

	N	Body weight (dry, mg)	Total fat (% of body weight)	TG (% of Total fat)
Female				
SW	22	208.0 $\pm$ 37.3	27.2 $\pm$ 6.7	45.1 $\pm$ 7.7
IM	5	203.2 $\pm$ 41.5	24.3 $\pm$ 5.3	44.2 $\pm$ 8.1
LW	24	233.7 $\pm$ 54.7*	28.0 $\pm$ 5.1	46.4 $\pm$ 7.8
Male				
SW	31	190.3 $\pm$ 35.3	20.9 $\pm$ 7.4*	44.0 $\pm$ 11.1
IM	6	210.6 $\pm$ 48.9	25.3 $\pm$ 8.3	43.4 $\pm$ 1.37
LW	16	212.2 $\pm$ 32.1	23.7 $\pm$ 4.6	44.4 $\pm$ 10.4

\* The mean followed by an asterisk is significantly different from the values of the other morphs of the same sex (Fisher's PLSD test;  $P < 0.05$ ).

total fat content, which was significantly lower than that of SW females or LW males ( $P < 0.001$  by Fisher's PLSD). Compared to the other morphs, IM morphs had a slightly small mean value in females and a large mean value in males (Table 3), but this would need to be confirmed with a larger sample size. Triacylglycerol (TG) content of the total fat was almost constant across the wing morphs (Fig. 8D; Table 3). These results indicated that lipid content of the body at adult emergence had no consistent wing-morph specific differences in this cricket.

#### Flight activity and formation of LDLp

To determine the time of flight, newly emerged LW adults were held in plastic tanks and the number of individuals leaving the tanks was recorded. They started leaving the tanks on day 3 (Fig. 9). The number of such individuals increased in the next 3 days and then no further change was seen. More than

50% of females left the tanks by day 4 while only 25% of males did so by the same day. They left the tanks mainly during the dark period, but some took off for flight during the light period. The latter were observed walking actively with their wings vibrating before flight. Most individuals found outside the tanks were collected every day and some dissected to observe the status of the flight muscles. With no exception, the flight muscles were well developed and dark pink in color ( $N = 10$ ).

To examine if AKH induces the formation of LDLp in both LW and SW morphs, 10 pmol Gb-AKH was injected into young adults and hemolymph samples collected 90 min later. The samples subjected to ultracentrifugation clearly showed that LDLp was formed in both morphs after injection of Grb-AKH, whereas the lipophorin of untreated controls remained as HDLp ( $N = 3$  each; data not shown). Because the presence of an AKH had not been confirmed for *T. derelictus*, methanol extracts of CC removed from SW and LW adults were injected into young LW adults to determine if the CC from both wing morphs would show AKH activity. LW adults injected with CC extracts from either morph formed LDLp in the hemolymph, and the yellow pigments showing the presence of lipophorin from such individuals were found at a more upper level on the density gradient than those from untreated LW or SW adults (Fig. 10). These results indicated that the CC from both wing morphs had AKH activity, and that both wing morphs could respond to injected Grb-AKH by mobilizing lipids into the hemolymph.

## Discussion

*T. derelictus* with continuous variation in wing

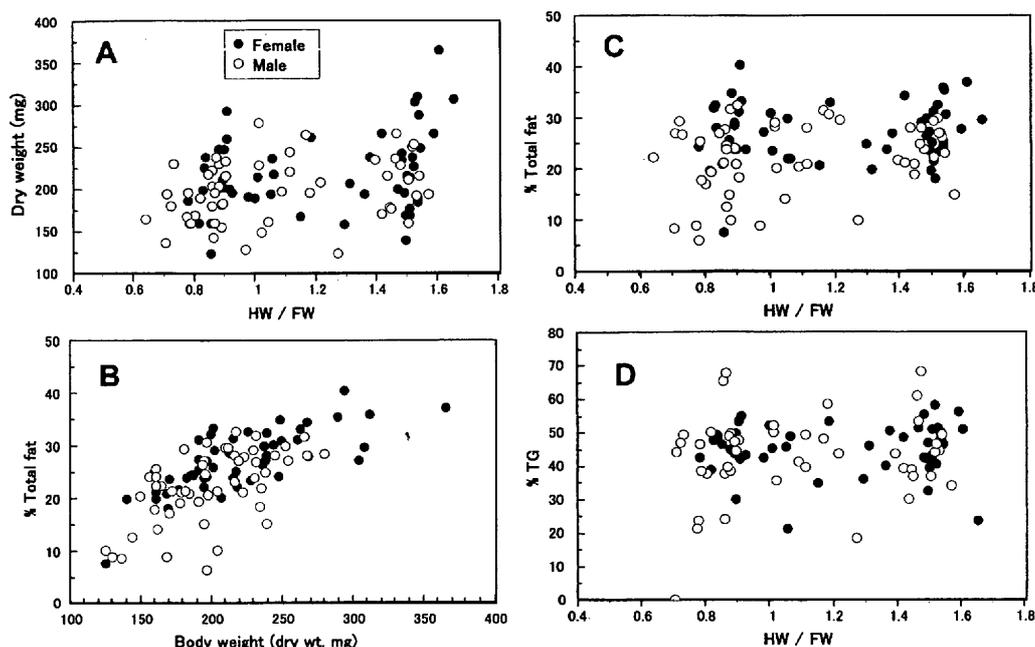


Fig. 8. Body weight and lipid contents of *T. derelictus* at adult emergence (N=51 and 53 in females and males, respectively). A, body weight vs. HW/FW,  $r=0.309$ ,  $P<0.05$  in females,  $r=0.283$ ,  $P<0.05$  in males; B, total fat content (% dry body weight) vs. body weight,  $Y=0.09X+7.575$ ,  $r=0.738$ ,  $P<0.001$  in females;  $Y=0.108X+0.693$ ,  $r=0.580$ ,  $P<0.001$  in males; C, total fat content vs. HW/FW,  $r=0.009$ ,  $P>0.05$  in females,  $r=0.186$ ,  $P>0.05$  in males; D, triacylglycerol (TG) content (% of total fat) vs. HW/FW,  $r=0.030$ ,  $P>0.05$  in females,  $r=0.186$ ,  $P>0.05$  in males.

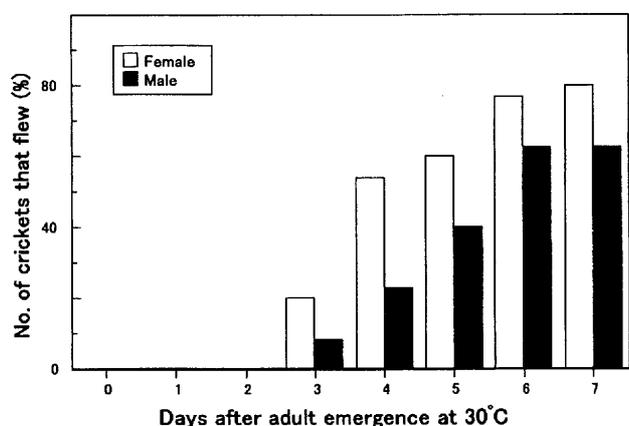


Fig. 9. Flight activity of LW morphs of *T. derelictus* during early adult life, as determined by the number of individuals that jumped out of the plastic tanks at 30°C. N=35 each. Newly emerged adults were held in the plastic tanks with food and the individuals that left the tanks were counted for 7 days at 30°C.

length provided a unique opportunity to examine several problems related to the evolution of wing dimorphism in insects. These problems include 1) the mechanism controlling the variation in wing length, 2) the status of flight muscle in various wing morphs, 3) flight capability and 4) related physiological adaptations.

Wing polymorphism is controlled by various factors in insects (Harrison, 1980). In crickets, both genetic and environmental factors such as photoperiod, tem-

perature, crowding and food are known to influence the determination of wing morph (Alexander, 1968; Masaki & Walker, 1987). In *T. derelictus*, the mechanism controlling wing length variation has not been studied. However, our preliminary experiment to test the effect of crowding on wing development has indicated that crowding may not be so important because all nymphs derived from a single pair of LW adults emerged as LW morphs irrespective of the rearing density (1, 2 and 10 individuals/container; S. Tanaka, unpublished data). On the other hand, 100% selection for SW morphs over 3 generations produced a substantial increase in the frequency of this morph (S. Tanaka, unpublished data), indicating that the genetic factor may be important for this cricket. However, systematical studies should be conducted to determine the exact mechanism controlling the wing length development of this cricket.

It is generally accepted that among the pterygote orders the winged condition is primitive and that reduction of wings (or flight muscles) has occurred independently in many different groups (Southwood, 1962; Vepsäläinen, 1978; Harrison, 1980). Anderson (2000) argued that the ancestral form of some water strider families was flightless and that dimorphic and monomorphic LW species evolved later. However, this scenario and the above statement are not mutually exclusive, because the ancestral heteropteran bugs

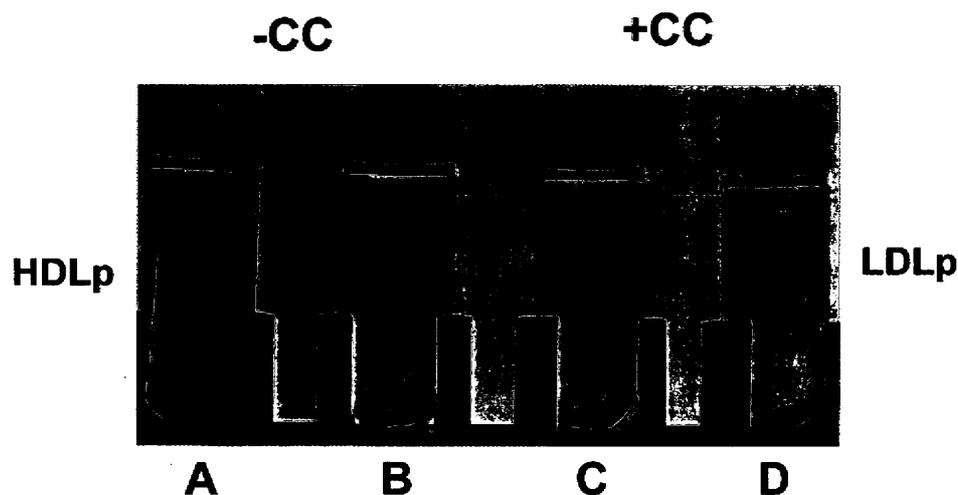


Fig. 10. Ultracentrifugation profiles on KBr of the hemolymph samples collected from LW adults in *T. derelictus* after injection of CC extracts. CC taken from SW and LW adults were extracted with methanol, and the supernatant of each extract was dried and dissolved in water for injection into 1-week-old LW adults. Injected crickets were incubated at 30°C for 90 min and bled to collect hemolymph samples which were then subjected to ultracentrifugation. Yellow pigments in the density gradient indicate the presence of lipophorin. High-density lipophorin (HDLp) was found in untreated LW (A) and SW morph (B), whereas lipid-loaded lipophorin or low-density lipophorin (LDLp) was observed in LW morphs (C and D) injected with extracts of CC taken from LW and SW adults, respectively.

were assumed to be the LW morph in Anderson's theory.

As mentioned earlier, Roff's hypothesis regarding the evolution of wing dimorphism is quite logical and may be supported by the presence of various dispersal polymorphisms. In a monomorphic LW species, *Oncopeltus fasciatus*, some individuals seldom fly over a long distance whereas others show migration by flight (Dingle, 1978), which may represent behavioral dimorphism as the first step in Roff's hypothesis. Species of Corixidae (Young, 1965; Scudder, 1971) and Scarabaeidae (Tada *et al.*, 1993, 1995) may fit the second step showing flight muscle dimorphism in the LW condition. In these species, individuals with the reduced flight muscle can not fly, although the wings are intact. The reduced wings and flight muscles, which coincide with the form in the third step of Roff's hypothesis, can be found in many wing dimorphic species including aphids (Johnson, 1953, 1957, 1959), bugs (Vepsäläinen, 1978; Tanaka & Wolda, 1987) and crickets (Tanaka, 1986a, b, 1993, 1994; Zera *et al.*, 1997).

The present results with *T. derelictus* do not support Roff's hypothesis, because this cricket shows continuous variation not only in wing length but also in flight musculature. In this species, modification of flight musculature apparently occurred concurrently with that of wing length or after the latter started. However, the close association between flight muscle mass and wing length in newly emerged adults of this

cricket seems to suggest the former scenario more likely. Continuous or more or less continuous variation in wing length has been reported in a cricket, *Grylloides supplicans* (Arai, 1978) and bugs, *Cavelerius saccharivorus* (Fujisaki, 1989) and *Pyrhocoris sibiricus* (Sakashita *et al.*, 1995), but the status of the flight muscles has not been investigated for those species. In another cricket, *Gryllus rubens*, Walker & Sivinski (1986) reported the presence of individuals with intermediate wing length, but the status of the flight muscle in such individuals was unknown. Our recent observations with *G. rubens* indicated that individuals with intermediate wing length developed intermediate sizes of flight muscles, as found in *T. derelictus*. Therefore, it seems that continuous variation in both wing length and flight musculature is not an exceptional phenomenon, indicating an alternative pathway for the evolution of wing dimorphism in insects.

The presence of intermediate morphs may indicate a close developmental association between flight muscle and wing development, which might have caused the occurrence of IM morphs with intermediate sizes of the flight muscle in *T. derelictus* and *G. rubens*. In this respect, it would be interesting to investigate the flight muscles in other species showing continuous variation in wing length.

The presence of intermediate sizes of wings and flight muscles is useless in terms of fitness, because they are not functional. Furthermore, since the energy resource required for construction of the flight

muscles is not available to other organs or tissues, we can easily imagine that natural selection will operate against such individuals. It is thus not unexpected that they were less common than the other morphs and that their flight muscle underwent rapid histolysis after adult emergence. Presumably, the nutrient derived from the flight muscle histolysis and the energy otherwise required for the maintenance of the muscles are used for development of other organs or for general maintenance of the body.

Flight muscle histolysis was also observed in some LW morphs in *T. derelictus*, but in other LW morphs the metathoracic muscles increased the dry mass by more than 70% during the first week of adulthood. In this case, the DLM mass approximately doubled. Flight muscle growth and histolysis after adult emergence are apparently common among LW morphs in other cricket species such as *Dianemobius mikado* (Tanaka, 1976), *Allonemobius fasciatus* (1986), *Acheta domesticus* (Novicki, 1989), *Teleogryllus oceanicus*, *G. firmus* (Roff, 1989), *Velarificturus parvus* (Tanaka, 1991), *Gryllus bimaculatus* (Gomi *et al.*, 1995) and *Modicogryllus confirmatus* (Tanaka, 1993, 1994a, b, 2001; Tanaka & Suzuki, 1998). Zera and his colleagues concluded that neither growth nor histolysis occurred in *Gryllus rubens* and *G. firmus* during the adult stage (Mole & Zera, 1993, 1994), but this conclusion appears to be incorrect according to other studies indicating that histolysis occurs in both species (Roff, 1989; Zera *et al.*, 1997) and growth in *G. rubens* (Tanaka, 2001; unpublished observations). In *T. derelictus*, the color of the flight muscle varies with the mass. It was whitish in SW morphs, pinkish in LW morphs and yellowish or light pink in IM morphs at adult emergence. In IM morphs, the DLM and DVM did not always assume the same color even in the same individual, but differed in darkness of the color at adult emergence (Fig. 6B). By the end of the first week of adulthood the histolyzed muscles of IM and LW morphs turned whitish, whereas the intact muscles in LW morphs became darker. A close correlation between the muscle color and the absorbance peak at about 410 nm may indicate that cytochrome *c* is responsible for the color change of the muscles.

LW morphs of *T. derelictus* become active after a few days of adult emergence. While SW adults remained in the rearing tanks with no cover, LW ones jumped out of the tanks during early adult life. We observed several LW adults taking off for flight during the photophase after displaying a typical pre-flight behavior: *i.e.* walking around actively with their hind wings vibrating, as reported for other crickets (Alexa-

nder, 1968; Tanaka *et al.*, 1999b). Therefore, LW morphs of this cricket are probably functional dispersers, although some LW individuals may histolyze the flight muscles and never fly. During a second excursion to Indonesia in 1997, LW and SW morphs were confirmed to occur on Sumatra Island (Arai, T. *et al.*, unpublished observations).

It is important for dispersers to store enough flight fuel before migration. In *Locusta migratoria*, gregarious adults store more triacylglycerol in the fat body than solitary ones, and the former can thus mobilize diacylglycerol (DG) in the hemolymph as a fuel in response to injection of CC extracts or synthetic AKH (Chino *et al.*, 1992; Ayali & Pener, 1992; Ayali *et al.*, 1994). Crickets also raise the hemolymph lipid concentration after injection of CC extracts or synthetic AKH (Gäde, & Rinehart, 1987; Woodring *et al.*, 1990; Cusinato *et al.*, 1991; Tanaka *et al.*, 1999b). However, no comparison has been made in the lipid mobilization capacity between flight-capable LW and flightless SW morphs. In the firebug, *Pyrhocoris apterus*, it has been reported that LW adults mobilized more lipids in the hemolymph in response to a locust AKH than SW adults, but this morph-specific difference is not related to flight capability because both wing morphs are flightless (Socha & Kodrik, 1999).

The present study demonstrated that the amount of fat or TG in the body depended on body size but not on HW/FW ratio. The lack of intermorph-difference in fat contents for newly emerged adults has also been reported for *M. confirmatus* (Tanaka *et al.*, 1999a). Feeding may increase fat content of the body during early adult life, as reported for *L. migratoria* (Tanaka & Okuda, 1996), *M. confirmatus* (Tanaka *et al.*, 1999a), and *N. lugens* (Itoyama *et al.*, 1999). Such an increase of fat (in mass) is particularly conspicuous in LW morphs of *M. confirmatus* (Tanaka *et al.*, 1999a). However, no comparable information has been available for feeding adults of *T. derelictus*.

The present study also demonstrated that both LW and SW morphs of *T. derelictus* can similarly respond to injected synthetic Grb-AKH by forming lipid-loaded LDLp in the hemolymph. Because the identity or even presence of the AKH was unknown for this cricket, we injected LW adults with methanol extracts of CC taken from LW and SW adults in order to determine if these extracts would show AKH activity. As a result, we confirmed that both morphs contained a factor(s) stimulating the formation of LDLp in the hemolymph. These results may indicate that the lipid mobilizing capability has been retained in the SW morph of *T. derelictus*, although there is the possibility

that some quantitative differentiation might have taken place between morphs through differences in AKH release or fat accumulation after adult emergence. In the present study, no information is provided concerning the capacity and efficiency of reproduction in different morphs. Studies to collect such information are in progress.

## Acknowledgments

We thank A. Ichikawa for identification of specimens of crickets and helpful information, T. Shiotsuki and T. Okuda (IIAS) for assistance with spectrophotometer, and N. Kemmochi, S. Ogawa and H. Ikeda (IIAS) for laboratory assistance. This study was carried out with the permission of The Indonesia Institute for Sciences (LIPI), and funded partly by Grants-in-Aid for International Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan to K. Nakamura (No. 08041141). Part of this study was conducted under the approval of the Institute of Low Temperature Science, Hokkaido University, Advisory Committee (Proposal No. 99-13). We are grateful to Dr. Sinzo Masaki (Emer. Prof. Hirosaki University) for encouragement and invaluable comments on the manuscript. Two anonymous referees improved the manuscript.

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(Received February 14, 2001; Accepted March 22, 2001)