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A Mediterranean population of *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae) exhibits wing morph-related differences in adipokinetic response*

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Abstract. Short-winged (brachypterous) and long-winged (macropterous) adult females of *Pyrrhocoris apterus* (L.) originating from a Mediterranean population (Israel) were analyzed for their adipokinetic responses. The adipokinetic response, expressed as an increase of haemolymph lipids after injection of adipokinetic hormone from *Locusta migratoria* (Lom-AKH-I), was assessed in relation to age and dose of the hormone. We demonstrate in this study that the adipokinetic responses induced by bug's corpora cardiaca extract and Lom-AKH-I were dose-dependent for both brachypterous and macropterous females. Significant differences between the morphs were recorded for doses ≥ 0.25 corpora cardiaca equivalent ($P < 0.01$) and doses ≥ 0.25 pmol Lom-AKH-I ($P < 0.05$). The haemolymph lipid elevations induced in both morphs by 2 pmols Lom-AKH-I are comparable with that induced by crude extract of one pair of the bug's own corpora cardiaca. The age-dependent test showed much higher adipokinetic responses ($P < 0.01$) in macropterous females (≥ 5 days old) than in the brachypterous females of the same age, when treated with 2 pmols Lom-AKH-I. Starting from day 7, the concentration of haemolymph lipids was also considerably higher (2.5–5 times) in macropterous females than in the brachypterous ones. The obtained data indicate that difference in mobilization of lipids between brachypterous and macropterous females is a geographically independent feature and represents a true wing morph characteristic of *P. apterus*.

INTRODUCTION

In contrast to the information on the biochemical aspects of adipokinetic hormones (AKHs) (see review Gäde, 1997), only a limited number of studies have shown the relationship between adipokinetic response and lipid content to phase polymorphism of insects. In locusts, e.g. in *Locusta migratoria migratorioides*, an occurrence of the AKH-related phase-dependent differences was shown to have a close relation to fuel utilization during flight and differences in migratory capacities of their gregarious and solitary phases (Ayali et al., 1996; Pener et al., 1997). The differences between the adipokinetic strategies of solitary and gregarious *Schistocerca gregaria* are not so conspicuous, nevertheless they also indicate a better adaptation of the gregarious phase to prolonged flights (Ogoyi et al., 1996; Schneider & Dorn, 1994). In addition, the phase-related differences in adipokinetic response were reported also for some noctuids (Fescemyer, 1993).

The relationship of adipokinetic response to wing dimorphism was first demonstrated in the flightless firebug, *Pyrrhocoris apterus* (L.) (Heteroptera: Pyrrhocoridae) (Socha & Kodrík, 1999). The authors found that the temperate population of this bug was typified by considerably higher adipokinetic responses in macropterous (long-winged) females than in the reproductive brachypterous (short-winged) ones, when reared under a long-day (18L : 6D) photoperiod. This difference was presumed to be a wing morph-related feature. Nevertheless, the *Pyrrhocoris* macropterous and brachypterous morphs were found to differ also in the length of pre-oviposition period (Socha & Šula, 1996), the content of hexameric storage protein (Socha & Šula, 1992; Šula et al., 1995), feeding and digestive enzyme activities (Socha et al., 1997, 1998), and lipid content in the fat body (Socha et al., 1998; Šula et al., 1998).

However, the question of whether the observed difference in adipokinetic responses of *P. apterus* represents a true wing morph characteristic of this bug or if it is a geographically dependent feature, will remain unanswered until realization of a similar comparative study with brachypterous and macropterous females of this bug from populations of different geographic origin. The present study was therefore focused to reveal the wing morph-related differences in adipokinetic response in females originated from a Mediterranean population of this bug.

MATERIAL AND METHODS

Experimental animals

A laboratory stock of the Mediterranean population of *P. apterus* (L.), originating from Israel, was reared on linden seeds and water ad libitum at a constant temperature of 26 ± 1°C under a long-day (16L : 8D) photoperiod. Larvae and adults were kept in 0.5 l jars in mass culture (approximately 40 specimens per jar), and food and water were replenished twice a week. Since the highest proportion of macropterous specimens (≈ 36%) is produced under a short-day (12L : 12D) photoperiod at constant temperature of 26 ± 1°C (Socha & Šula, 1996), the bugs destined for analyses of adipokinetic responses were reared from the eggs until adult stage under these photoperiodic and temperature conditions.

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Two different experimental groups, i.e. brachypterous and macropterous females, were used in the present study. Small groups (10–20 individuals) of freshly ecdysed adult females were transferred into small glass jars (250 ml) and kept under the same photoperiodic and temperature regimes in which they had developed. The bugs were supplied with linden seeds and water in small glass tubes plugged with cellulose wool.

Hormonal treatments

The Lom-AKH-I from *Locusta migratoria* (Calbiochem) and methanolic extract from the corpora cardiaca of the 3-day-old reproductive females of *P. apterus* were used in our experiments. The reasons for the choice of the Lom-AKH-I were explained elsewhere (Socha & Kodrík, 1999). In short, this hormone exhibited the highest adipokinetic activity among several tested AKHs; the adipokinetic response of the hormone is comparable to the same response of the crude bug's own corpora cardiaca extract; and the hormone is easily and commercially available in sufficient amounts needed for experiments.

The extract from corpora cardiaca was obtained using 80% methanol: the dissected organs were sonicated, centrifuged and the supernatant was evaporated to dryness. The rest was dissolved in 20% methanol in Ringer saline and used for injections. Stock solutions of the hormone were prepared also in the methanol-Ringer saline (0.05–5 pmol AKH/μl) and stored as aliquots at –20°C. Peptide dilutions were prepared to give the desired amount of the peptide per 2 μl solution. This volume was injected with the help of a syringe (5 or 10 μl; Hamilton Co., Reno, Nevada) through the metathoracic-abdominal intersegmental membrane to the thorax of the experimental bug. Control bugs were injected with 2 μl of 20% methanol in Ringer saline in the same way.

There were two main reasons for using the 10-day-old females in the adipokinetic dose-response tests of the corpora cardiaca extract and the Lom-AKH-I: the response was maximal in brachypterous females of this age category (see Results) and the data were comparable with those obtained for females of two wing morphs that originate from the temperate population (Socha & Kodrík, 1999).

Haemolymph sampling and bioassay

Lipid concentration in the haemolymph samples was determined by a method based on the sulpho-phosphovanilin test (Zöllner & Kirsch, 1962; modified by Holwerda et al., 1977 and Van Marrewijk et al., 1984). The haemolymph samples were taken from an antenna that was cut off by fine scissors. A drop of haemolymph was leaked onto a piece of parafilm M. An aliquot of 0.5 μl haemolymph was taken by a special micropipette (Eppendorf Varipipette 4810) from the drop and used for the determination of lipids. The samples were collected just before and 90 min after the injection. The optical densities, recorded at 546 nm in a spectrophotometer (Pye Unicam 1800), were converted with the aid of a calibration graph, based on known aliquots of oleic acid to mg lipids per ml haemolymph using the Microsoft Excel program. Results were expressed as a mean (n = 5–14) of either haemolymph lipid elevation (difference of lipid levels after and before injection) or haemolymph lipid concentration ± SD.

Data presentation and statistical analyses

The results of adipokinetic responses were plotted and the curves were fitted by means of the program Fig. P (Biosoft, version 6.0, Cambridge, U.K.). For statistical analyses an unpaired t-test with the help of the program Instat (GraphPad Software, version 1.15, San Diego, CA, U.S.A.) was used.

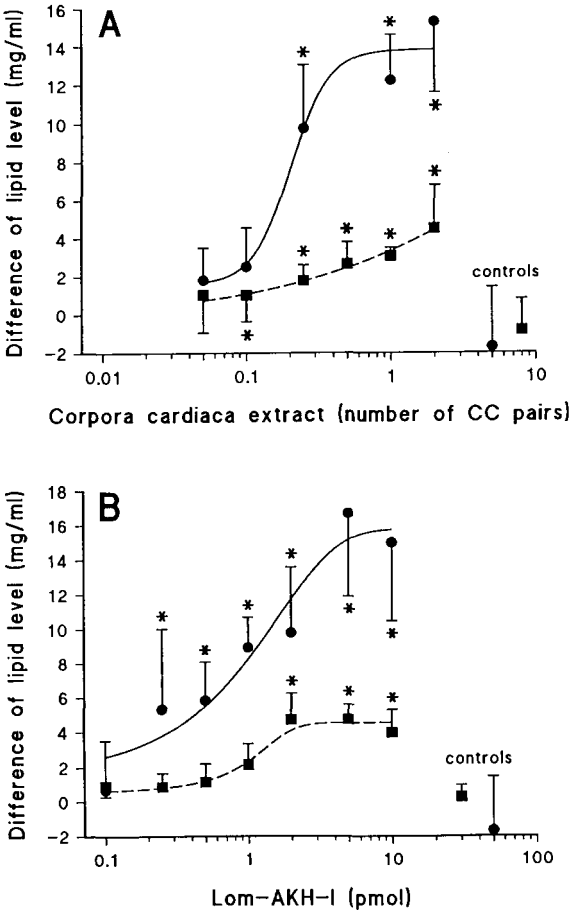

Fig. 1. Effect of increasing doses of the crude extract of corpora cardiaca (CC) (A) and Lom-AKH-I (B) on elevation of haemolymph lipid level in brachypterous (--■--) and macropterous (–●–) females of *P. apterus* at day 10 of their adult life (A: n = 5–8, B: n = 5–14). Controls represent effect of injected saline. Statistically significant differences at the 5% level are indicated by * (experimental vs. control).

RESULTS

The dose-response tests of the corpora cardiaca extract and the Lom-AKH-I on the 10-day-old females of brachypterous and macropterous morphs revealed that haemolymph lipid elevations induced by both the corpora cardiaca extract (from 3-day-old reproductive females) (Fig. 1A) and the Lom-AKH-I (Fig. 1B) were dose-dependent for both wing morphs. A comparison of the obtained data between the morphs revealed significantly lower adipokinetic responses in brachypterous females than in macropterous ones for doses ≥ 0.25 pair of the corpora cardiaca equivalent ($P < 0.01$) and doses ≥ 0.25 pmol Lom-AKH-I ($P < 0.05$), respectively.

In the age-dependent test (Fig. 2) we used a dose of 2 pmol of the hormone. The effect of this dose was comparable to that of 1 pair of the corpora cardiaca extract (Fig. 1). Freshly ecdysed females of both experimental groups were not responsive to the hormone (Fig. 2), but the adipokinetic response increased with age and became significant (experimental vs. control, $P < 0.05$) from the 5th (brachypters) and 3rd (macropters) day respectively. The results confirmed the previous data from the dose-

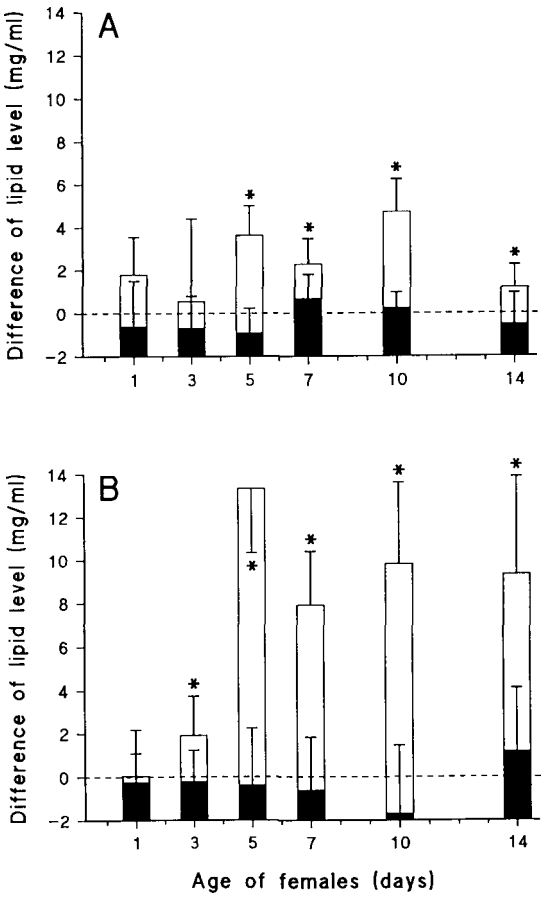

Fig. 2. Effect of 2 pmol Lom-AKH-I (□) with corresponding effect of injected saline (■) on the elevation of haemolymph lipid level in brachypterous (A) and macropterous (B) females of *P. apterus* at different time after adult ecdysis (A: n = 6–11, B: n = 6–14). Statistically significant differences at the 5% level are indicated by * (experimental vs. control).

response test: the adipokinetic response was significantly lower ($P < 0.01$) in brachypterous females than in macropterous ones for the age category from the 5th to 14th day. The very dramatic elevation of the adipokinetic response in macropterous females recorded between the 3rd and the 5th day of their adult life was probably associated with a cessation of food intake.

The different strategy in lipid mobilization in the bugs of the brachypterous and macropterous morphs is also documented by different changes of lipid concentration during adult life (Fig. 3). The haemolymph lipid concentration increased with the age of adult females until the

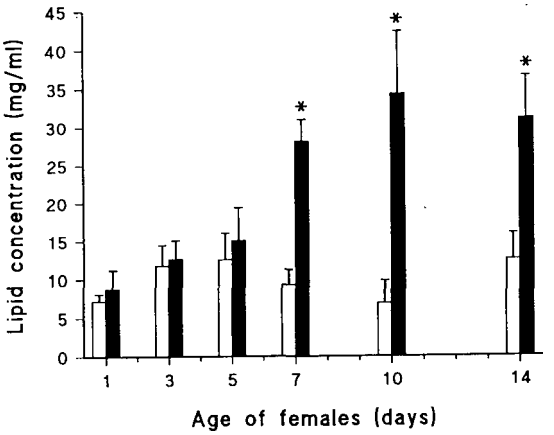

Fig. 3. Temporal course of the changes in haemolymph lipid concentration in brachypterous (□) and macropterous (■) females of *P. apterus* during the first 14th days of their adult life (n = 6–14). Statistically significant differences at the 1% level are indicated by * (brachypterous vs. macropterous).

5th day – both experimental groups exhibited very similar data pattern during the period. Later, the lipid concentration dramatically increased in macropterous females and reached its maximum on the 10th day (5 fold higher concentration than in brachypters), while it decreased or stayed more or less stable in brachypterous individuals. Significant differences ($P < 0.01$) were recorded between both groups within the 7th–14th day of adult life.

DISCUSSION

The present results showed the Mediterranean population of *P. apterus* to be characterized by a markedly higher adipokinetic response and haemolymph lipid content in macropterous females than in brachypterous ones. Similar findings were obtained also for females of two wing morphs originating from the temperate population of this bug reared under a long-day (18L : 6D) photoperiod (Socha & Kodrík, 1999). Similar results obtained for females originating from two geographically different populations of *P. apterus* (for comparison see Table 1) thus strengthen the authenticity of our hypothesis that the wing morph-related difference in adipokinetic responses of this bug represents a specific wing morph characteristic of this species.

Similarly, the geographically independent wing morph-related differences were demonstrated also in the concentration of 78- and 82-kDa polypeptides in the haemolymph of *P. apterus* females (Socha & Šula, 1996).

TABLE 1. Comparison of the effect of 2 pmol Lom-AKH-I and crude extract of the corpora cardiaca from 3-day-old female on elevation of haemolymph lipid level at 10-day-old females of *P. apterus*.

Experimental group	Increase of lipids (mg/ml) (n)		
	Lom-AKH-I	1 CC pair	Control
M-br	4.73 ± 1.53* (8)	3.08 ± 0.45* (5)	−0.82 ± 1.63 (6)
M-ma	9.82 ± 3.79* (14)	12.20 ± 2.36* (8)	−1.68 ± 3.13 (5)
T-br[1]	6.07 ± 2.21* (17)	4.32 ± 1.22* (7)	0.60 ± 1.53 (11)
T-ma[1]	9.97 ± 1.64* (9)	9.84 ± 2.17* (8)	0.31 ± 3.25 (5)

Abbreviations: M – Mediterranean population; T – temperate population; br – brachypterous females; ma – macropterous females; CC – corpora cardiaca extract.

* $P < 0.05$ (experimental vs. control); [1]data taken from Socha & Kodrík (1999).

An absence of these storage proteins in the macropterous females with reproduction arrest of non-diapause type (in contrast to a conspicuous accumulation of these proteins in diapausing brachypterous females) appears to be another specific wing morph-related trait of *P. apterus*, being independent of the geographical origin of this bug.

In contrast to the reproductive and diapausing brachypterous females of *P. apterus*, the dispersal activity of macropterous females of this bug is associated with drastic inhibition of feeding and reproduction, and coupled with enhanced mobilization of lipid reserves from the fat body (Socha et al., 1998; Šula et al., 1998). The lipids provide a substantial part of energy demands in macropterous females of this bug, since glycogen content in their fat body is very low, 20% of glycogen in reproductive brachypterous females, and 4% of glycogen in diapausing brachypterous females on day 14 of their adult life (Šula et al., 1998). As the lipid content in the fat body is lower in macropterous than in brachypterous females (Šula et al., 1998), the reduced AKH response in brachypterous females appears to be conditioned rather by lower efficacy of this hormone in these individuals. All these data are in accordance with an occurrence of wing morph-related differences in adipokinetic response of *P. apterus*. The higher levels of haemolymph lipids (present results) and the more intensive process of lipid mobilization from the fat body in macropterous females of this bug during their reproductive arrest (Socha et al., 1998) might represent the metabolic relics conserved from their flying ancestors. The aim of the intense lipid mobilization in recent macropterous individuals of this species is to ensure their enhanced locomotor and dispersal activities even when flight was substituted with running (Socha et al., 1999). This assumption is supported by our recent findings that the non-functional (flightless) macropterous morph of contemporary populations of *P. apterus* maintains its dispersive nature and differs from the brachypterous morph by higher locomotor activity and walking path characteristics (walking speed, turning rate, sinuosity etc.) (Socha & Zemek, unpubl. data).

In summary, two wing morphs of *P. apterus* can be distinguished by specific behavioural, biochemical and physiological features, including the differences in their adipokinetic response. The wing morph-related difference in adipokinetic response of *P. apterus* was shown to be a geographically independent physiological marker specific for this heteropteran species, and probably associated with different roles of two wing morphs in the life strategy of this bug. We suppose that the wing morph-related differences in adipokinetic response might represent a more general feature in insects whose wing morphs differ by flight capability and use lipids as the main energy fuel for migration of macropters.

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REFERENCES

Ayali A., Golenser E. & Pener M.P. 1996: Flight fuel related differences between solitary and gregarious locusts (Locusta migratoria migratorioides). *Physiol. Entomol.* **21**: 1–6.

Fescemyer H.W. 1993: Influence of phase polymorphism on the morphometric and physiological processes in preparation for insect migration. *J. Agr. Entomol.* **10**: 219–237.

Gäde G. 1997: The explosion of structural information of insect neuropeptides. In Herz W., Kirby G.W., Moore R.E., Steglich W. & Tamm Ch. (eds): *Progress in the Chemistry of Organic Natural Products 71*. Springer Verlag, New York, pp. 1–128.

Holwerda D.A., Van Doorn J. & Beenakkers A.M.T. 1977: Characterization of the adipokinetic and hypertrehalosaemic substances from the locust corpus cardiacum. *Insect Biochem.* **7**: 151–157.

Ogoyi D.O., Osir E.O. & Olembo N.K. 1996: Effect of phase status on responses to AKH I in the desert locust, Schistocerca gregaria. *Arch. Insect Biochem. Physiol.* **32**: 173–185.

Pener M.P., Ayali A. & Golenser E. 1997: Adipokinetic hormone and flight fuel related characteristics of density-dependent locust phase polymorphism: A review. *Comp. Biochem. Physiol. (B)* **117**: 513–524.

Schneider M. & Dorn A. 1994: Lipid storage and mobilization by flight in relation to phase and age of Schistocerca gregaria females. *Insect Biochem. Mol. Biol.* **24**: 883–889.

Socha R. & Kodrík D. 1999: Differences in adipokinetic response of Pyrrhocoris apterus (Heteroptera) in relation to wing dimorphism and diapause. *Physiol. Entomol.* **24**: 278–284.

Socha R. & Šula J. 1992: Voltinism and seasonal changes in haemolymph protein pattern of Pyrrhocoris apterus (Heteroptera: Pyrrhocoridae) in relation to diapause. *Physiol. Entomol.* **17**: 370–376.

Socha R. & Šula J. 1996: Differences in haemolymph proteins in relation to diapause and wing dimorphism in Pyrrhocoris apterus (L.) (Heteroptera: Pyrrhocoridae). *J. Comp. Physiol. (B)* **166**: 382–387.

Socha R., Šula J. & Zemek R. 1997: Feeding, drinking and digestive enzyme activities in long- and short-day females of Pyrrhocoris apterus (Heteroptera). *Physiol. Entomol.* **22**: 161–169.

Socha R., Šula J. & Zemek R. 1998: Feeding behaviour, digestive physiology and lipid content in macropterous females of Pyrrhocoris apterus (L.) (Heteroptera: Pyrrhocoridae). *Physiol. Entomol.* **23**: 91–96.

Socha R., Kodrík D. & Zemek R. 1999: Adipokinetic hormone stimulates insect locomotor activity. *Naturwissenschaften* **88**: 85–86.

Šula J., Kodrík D. & Socha R. 1995: Hexameric haemolymph protein related to adult diapause in the red firebug, Pyrrhocoris apterus (L.) (Heteroptera). *J. Insect Physiol.* **41**: 793–800.

Šula J., Socha R. & Zemek R. 1998: Wing morph-related physiological differences in adults of temperate population of Pyrrhocoris apterus (L.) (Heteroptera). *Comp. Biochem. Physiol. (A)* **121**: 365–373.

Van Marrewijk W.J.A., Van den Broek A.T.M., Van der Horst D.J. & Beenakkers A.M.T. 1984: Hypertrehalosaemic and hyperlipaemic responses to adipokinetic hormone in fifth larval instar locusts, Locusta migratoria. *Insect Biochem.* **14**: 151–157.

Zöllner N. & Kirsch K. 1962: Über die quantitative Bestimmung von Lipoide (Mikromethode) mittels der vielen natürlichen Lipoiden (allen bekannten Plasmalipoiden) gemeinsamen Sulfo-phospho-vanillin-Reaktion. *Z. Ges. Exp. Med.* **135**: 545–561.

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