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Total Lipid and Fatty Acid Composition in Male and Female Larvae of Indian-Meal Moth and Almond Moth (Lepidoptera: Pyralidae)

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TOTAL LIPID AND FATTY ACID COMPOSITION IN MALE AND FEMALE LARVAE OF INDIAN-MEAL MOTH AND ALMOND MOTH (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

The total body lipid and fatty acid composition of last instar larvae of the Indian-meal moth, *Plodia interpunctella*, and almond moth, *Cadra cautella*, reared on a turkey mash diet was determined. Male *P. interpunctella* larvae contained significantly higher (1.4-fold) total body lipid than females, while no differences between the sexes of *C. cautella* larvae were observed. The relative abundance of the fatty acids palmitate, palmitoleate, stearate, oleate, lineoleate, and linolenate was similar in both sexes of *P. interpunctella* and *C. cautella*. The accumulation of individual fatty acids in larvae of both the moth species did not correspond to levels of fatty acids in the diet. The accumulation of palmitate, palmitoleate, and oleate in moth larvae of both the species was greater than linoleate and linolenate, suggesting a sparing effect by the former on the latter, more unsaturated fatty acids.

Lipid composition is influenced by sex during development in some Lepidoptera (Gilbert 1967). For example, fourth instar larvae of male silkworm, *Bombyx mori* (L.), (Niemierko et al. 1956) and fifth instar larvae of male Oak silkworm, *Antherea pernyi* Guerin, (Demyanovsky and Zubova 1957) contained higher lipid reserves than females of similar age. A sexual dimorphism in the requirement of lipids was reported in seven families of Lepidoptera (Gilbert 1967). Turunen (1974) reported a sexual dimorphism in the requirement of a polyunsaturated fatty acid (linolenate) in the cabbage butterfly, *Pieris brassicae* (L.). The adaptive significance of sexual dimorphism in lipid content in larvae is not clearly understood, but the factors producing such differences may be genetically determined (Gilbert & Schneiderman 1961). Though most immature male Lepidoptera contain more lipid than females, the utilization of these reserves during pupal-adult transformation is greater in females than males (Gilbert 1967, Downer 1978).

The total body lipid in last instar larvae of male Indian-meal moth, *Plodia interpunctella* (Hübner), was greater than in females (Yurkiewicz 1969). However, Yurkiewicz (1969) did not show whether the differences were significant. Worthington and Payne (1974) reported the fatty acid composition in larvae of *P. interpunctella* and the almond moth, *Cadra cautella* (Walker). However, they did not mention the stage or sex of the larvae.

Therefore, this study was undertaken to determine the total body lipid and principal fatty acid composition of last instar male and female larve of *P. interpunctella* and *C. cautella*. Principal fatty acids studied were palmitate, palmitoleate, stearate, oleate, linoleate, and linolenate. These are the fatty acids most commonly occurring in Lepidoptera (Fast 1964, 1966), and constitute 98% or more of the total fatty acids in larvae of *P. interpunctella* and *C. cautella* (Worthington and Payne 1974).

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MATERIALS AND METHODS

Plodia interpunctella and *C. cautella* were reared in the laboratory on a diet consisting of turkey mash (1000 g), honey (150 ml), glycerin (150 ml), and distilled water (75 ml). Eggs laid within 24 h of adult collection were used for rearing at 26–27°C and 50% RH.

Total body lipid was extracted and purified from live, 16-day-old, last instar male and female larvae of *P. interpunctella* (5th instar) and *C. cautella* (4th instar) in 2:1 chloroform:methanol mixture according to the procedure of Folch et al. (1957). Female larvae were identified by the absence of testes visible through the integument of males. Five groups of 20 male or female larvae of each species were extracted (five determinations/species/sex), and the total body lipid was expressed as per cent of live body weight.

Fatty acid methyl esters were prepared by evaporating 0.5 ml volume of chloroform containing lipid under nitrogen, and methylating by addition of 5 ml of benzene:boron trifluoride in methanol (12% w/v): methanol [1:1:1, (v/v/v)] mixture (Beenakkers and Gilbert 1968). The mixture was heated for 90 min at 98°C in 40 ml teflon-capped glass centrifuge tubes. The tubes were cooled, and 3 ml of water and 4.5 ml of hexane added and vigorously shaken. The upper hexane layer was removed, and the remaining mixture reextracted with 5.5 ml of hexane. The combined hexane fraction containing fatty acid methyl esters was taken up in isooctane, after evaporation of the hexane under nitrogen. Fatty acid methyl esters were analyzed using a Hewlett-Packard gas chromatograph (Model 5830 A) equipped with a flame ionization detector. The conditions of chromatography were as follows: glass column of 30.48 cm length with an internal diameter of 2 mm and packed with 79.SP 2330 on Chromosorb A-AW 100/200 mesh size; nitrogen carrier gas flow rate = 20 cc/minute; initial temperature = 160°C; initial hold = 4 min; program rate = 10°C/min; final temperature = 200°C; final hold = 7 min; injection temperature = 280°C; and detector temperature = 350°C. Individual fatty acid peaks were identified by reference to a standard mixture of fatty acid methyl esters (e.g. FAME MIX- 28 & FAME MIX-38, Alltech Associates), which were subjected to gas chromatography under the same conditions as were the insect samples. Three samples of each sex/species were injected. Each sample was injected twice and the average value considered. Relative abundance of individual fatty acids was computed by area normalization (McNair & Bonelli 1969). Fatty acids in the moth diet were prepared and analyzed in a similar manner from 1 g of diet.

Difference between sexes in the total body lipid and each fatty acid for each species was determined using Wilcoxon's two-sample signed rank test at $P = 0.05$.

RESULTS AND DISCUSSION

Male larvae of *P. interpunctella* contained significantly ($P < 0.05$) higher (1.4-fold) total body lipid than female larvae, while there was no difference ($P > 0.05$) between the larval sexes of *C. cautella* (Table 1). Yurkiewicz (1969) found higher levels of neutral lipid per gram live weight in male, last instar *P. interpunctella* larvae than female larvae. However, he did not indicate whether these differences were significant. A major portion of the total lipid in most Lepidoptera (Fast 1964, 1966; Gilbert 1967), and in these two moth larvae is in the triglyceride form (Yurkiewicz 1969, Worthington and Payne 1974). Sexual dimorphism in lipid content was evident in seven families of Lepidoptera (Gilbert 1967). The lack of any sexual dimorphism in total lipid content in *C. cautella* larvae suggests that these differences may be genetically controlled (Gilbert and Schneiderman 1961).

There was no difference ($P > 0.05$) between the sexes of *P. interpunctella* or *C. cautella* in the individual fatty acid composition (Table 2). The most abundant fatty acid in *P. interpunctella* was oleate, followed by palmitate, palmitoleate, linoleate, stearate, and linolenate. In *C. cautella* larvae, palmitate was the most abundant fatty acid followed by oleate, linoleate, stearate, palmitoleate, and linolenate. However, in the moth diet, the

Table 1. Total body lipid (mean % of live weight [S.E.]) of last instar larvae of male and female *P. interpunctella* and *C. cautella*.[a]

	P. interpunctella		*C. cautella*	
	Male	Female	Male	Female
No. larvae used	95	98	100	100
Total lipid	16.8 (0.8)	12.3 (0.4)	13.5 (0.4)	14.1 (0.1)
P-value	0.03[b]		0.43	

[a]Each mean based on five replicates.
[b]Significant ($P < 0.05$, by Wilcoxon's two-sample signed rank test).

Table 2. Relative abundance (mean % of total [S.E.]) of principal fatty acids in last instar larvae of male and female *P. interpunctella* and *C. cautella*.[a]

Fatty acid		*P. interpunctella*[b]			*C. cautella*[b]			Moth diet
		Male	Female	*P*-value	Male	Female	*P*-value	
Palmitate	(16:0)[c]	31.8 (0.5)	31.3 (0.2)	0.42	40.7 (0.7)	41.8 (0.04)	0.14	14.9 (0.9)
Palmitoleate	(16:1)	12.2 (0.7)	13.2 (0.6)	0.25	3.6 (0.8)	4.5 (0.4)	0.25	0.4 (0.1)
Stearate	(18:0)	5.2 (2.8)	2.0 (0.5)	0.25	5.0 (0.4)	3.2 (0.7)	0.14	4.8 (1.7)
Oleate	(18:1)	41.2 (1.0)	40.8 (0.4)	0.68	37.8 (1.7)	36.6 (0.4)	0.68	23.1 (0.5)
Linoleate	(18:2)	8.9 (1.2)	11.5 (0.6)	0.14	10.2 (0.3)	11.6 (0.7)	0.14	53.8 (3.1)
Linolenate	(18:3)	0.8 (0.2)	1.2 (0.1)	0.14	2.6 (0.1)	2.3 (0.2)	0.14	3.3 (0.3)

[a]Each mean based on three replicates.
[b]For each species, differences between sexes are not significant ($P > 0.05$, by Wilcoxon's two-sample signed rank test).
[c]Number of carbons: Number of double bonds.

abundant fatty acids in descending order were as follows: linoleate > oleate > palmitate > stearate > linolenate > palmitoleate (Table 2). Worthington and Payne (1974) reported low levels of palmitoleate, stearate, and linolenate in *P. interpunctella* and *C. cautella* larvae.

The accumulation of oleate in both moth larvae was about 2-fold greater than that present in the diet. Linoleate was about 5-fold greater in the diet (53.8%) but very little was accumulated by the moth larvae (9–11%). Polyunsaturated fatty acids, especially linoleate and linolenate, are essential for normal growth, development, and adult emergence of *P. interpunctella* and *C. cautella* (Fraenkel and Blewett 1946). The levels of individual fatty acids in the larvae did not correspond to levels in the diet. Nevertheless, the greater accumulation of palmitate, palmitoleate, and oleate than linoleate or linolenate suggests a sparing effect by the former mono-saturated and -unsaturated fatty acids on the latter polyunsaturated acids (Downer 1978, Turunen 1983). An increase in monoenoic acids was observed in larvae of *P. brassicae* reared on linolenate-deficient diets (Turunen 1974, 1983). Our observations on the compositions of palmitate and linolenate in both moth larvae agree with results obtained by Worthington and Payne (1974).

Differences in the total body lipid and principal fatty acid composition between sexes and species may be related to their lipid requirements. The levels of lipids and fatty acids are profoundly influenced by the diet (Barlow 1966, Turunen 1983). Since the supporting diet used for *P. interpunctella* and *C. cautella* was the same, variations in lipid components reflect basic physiological and metabolic differences between the species.

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