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LIPID CONTENT AND ENERGETIC EQUIVALENT AS A MEASURE OF REPRODUCTIVE EFFORT IN TWO SPECIES OF *TRIBOLIUM*

ABSTRACT: Comparison was made between two ways of estimation of reproductive effort using relationships between progeny and the parental female. Both lipid content and energetic value yield consistent results of reproductive effort differences both between instar groups and between different species. The cost of maturation and eclosion was calculated as based on the loss of energy or lipid content. This cost is higher in 7-instar groups (over 55%) and it is true for both sexes in the two species.

KEY WORDS: lipid content, energetic value, reproductive effort, *Tribolium*, intrapopulation diversity.

1. INTRODUCTION

The ratio of lipid content or energetic value of offspring to the body weight of a female or its energetic value has been often used to measure the reproductive effort in animals (Tinkle and Hardley 1975, Clarke 1977, 1979, Grahame 1977, Grant 1990, Todd and Havenhand 1990, Willows 1990, Donnelly and Guyer 1994). It depicts investment of an organism in its progeny. Other measures were also used to describe the reproductive effort such as ratio of clutch size and clutch weight to the body weight. In the case of *Tribolium*, a continuous egg laying brings about that daily egg production is the most convenient quantity for calculation of the proportion mentioned.

According to Tinkle and Hardley (1975), the best way to estimate relative reproductive effort is finding proportion between the total energy consumed by an organism to that allocated in reproduction. The latter approach will be developed in a more extensive paper compiling data of the present study and energy budgets of *T. castaneum* Hbst. cI strain (Klekowski et al. 1967), and of *T. confusum* Duval bIV strain (Bijok 1989a).

The aim of this study is to test differences in reproductive effort between the 6- and 7-instar groups discerned earlier (Prus 1976, Bijok 1986) in the above mentioned strains.

2. MATERIAL AND METHODS

The compared strains were cI of *Tribolium castaneum* (Herbst) and bIV of *T. confusum* Duval. Within each of these strains, 6- and 7-instar groups have been distinguished in our laboratory and kept as a separate substrains.

The collected material for adults, pupae and eggs in 6- and 7-instar groups of the both species were dried at a constant temperature of 60 °C. Then it was homogenised in a mortar. A part of material that was meant for lipids determination was weighted into aliquots. From these samples lipids were extracted by a mixture of chloroform, methanol and naphtha ether. After extraction the lipids were weighed and its percentage in dry matter content was calculated.

Another part of the material was used for determination of energetic value by forming pellets and combusting them in the Phillipson microbomb calorimeter (Prus 1975). The obtained results were expressed in joules per mg dry weight. The lipid content and energetic value were also determined in the culture medium consisting of 95% of wheat flour and 5% of powdered baker's yeast. The culture for collecting eggs, pupae and adults were run at 29 °C, 75% RH in a dark incubator.

The eggs taken for analysis were 0 to 24 hrs old and pupae separated into males and females, were taken on the third day of the pupal stage duration. This was meant to make the material as comparable as possible since the energetic value of both the eggs and pupae diminish along the development process.

The females and males of adults were originated from synchronised age cultures of the two species separated into 6- and 7-instar groups on the basis of differences in pupal appearance differences (Bijok 1989b). The sex of pupae has been determined and both males and females were placed separately in vials with medium. To the vial with males about 5% of females was added, same was done for females. Such procedure did not affect substantially the values of lipid content and energetic equivalent calculated for male and female, but it allowed animals to mate. Animals were kept in this vials for ten days. Later on, the animals were killed with the temperature shock and dried at 60 °C.

Students t-test was applied to assess significance between species/groups/ /sexes differences.

3. RESULTS

The results on lipid contents in eggs, pupae and adults of *Tribolium* revealed a very high differentiation of this trait. In general, the lipid share in dry weight of eggs was much lower, around 7 to 10%, whereas that in pupal stages ranged from about 37 to 51%. Lipid content in mature males and females of the two groups and species examined ranged from about 20 to 30% (Table 1). Percentage of lipids in *T. castaneum* eggs was slightly but consistently lower than that in *T. confusum*, especially in 6-instar groups this difference is significant. It was also lower in both the 6-instar groups than in 7-instar groups. That difference is larger within *T. castaneum* with probability of $p < 0.05$. Thus we know that *T. confusum* lays fatter eggs than *T. castaneum*. Eggs laid by females representing 7-instar groups are fatter than 6-instar groups.

Table 1. Lipid contents in dry matter (%) of *Tribolium* (mean values ± SD)
Lipid content in food (wheat flour + yeast) – 4.05%

Stage	*T. castaneum* cI		T. confusum bIV	
	6-instar	7-instar	6-instar	7-instar
Egg	7.50 ± 0.32	9.90 ± 1.54	9.44 ± 0.97	10.39 ± 0.44
Pupae (males)	38.64 ± 2.45	37.27 ± 3.99	42.53 ± 1.62	51.21 ± 0.48
Pupae (females)	37.93 ± 2.08	39.46 ± 3.37	39.02 ± 2.98	46.71 ± 0.42
Adult (males)	23.30 ± 0.73	19.50 ± 2.69	29.30 ± 0.28	28.12 ± 1.31
Adult (females)	21.08 ± 0.23	19.48 ± 1.12	19.60 ± 0.39	19.26 ± 0.48

The resources of lipids accumulated in pupae are rather of a crucial importance for the further life of adults. In *Tribolium* there are rather long periods of non-feeding stages (prepupae, pupae) amounting one fifth or one sixth of the whole developmental cycle, before the callow beetles start feeding again. High content of lipids in pupae is a convenient situation for seeking differences between the discerned groups. Pupae of *T. confusum* show generally higher lipid content then those of *T. castaneum* (39–51% versus 37–39%, respectively), the difference is significant at $p < 0.002$ (except 6-instar females). In the former species males show higher lipid percentage than do females (by about 10%), whereas in *T. castaneum* the differentiation in lipid content is much lower. In general the lipid content is higher in 7-instar group than in 6-instar group (only in *T. confusum* males that difference is nonsignificant). The lipid content in the food is rather low and it amounts to 4.0% of dry weight (Table 1). Lipid content in *T. castaneum* males is lower than that of *T. confusum* males ($p < 0.002$). The contrary situation is observed in females, but only in 6-instar group difference is significant. Similarly as in pupae, lipid content in adult females is higher than in males ($p < 0.001$ except 7-instar *T. castaneum*). 6-instar group has lower lipid content in adults' bodies than 7-instar one.

Energy equivalents (J mg dry wt^{-1}) point to a very uniform energy value of eggs in two groups of both species (Table 2). These values are rather low, lower by 6.0 J mg dry wt^{-1} than that of pupae. Energetic value of *T. confusum* pupae is higher than that of *T. castaneum*. The trend is the same in all examined groups however differences are slightly below the level we can consider as significant. The opposite trend can be observed in that value in adult females: energetic equivalent in *T. castaneum* exceeds that in *T. confusum* ($p < 0.002$). The energetic value of males is a little higher than that of females in *T. castaneum* and considerably higher in *T. confusum*, the latter being reflection of much higher lipid content in *T. confusum* males. The energy equivalent of insect's food is 17.2 J mg dry wt^{-1}.

Table 2. Energetic value (J mg dry wt^{-1}) in *Tribolium* (mean values ± SD)
Energetic value of food – 17.2 J mg dry wt^{-1}

Stage	*T. castaneum* cI		*T. confusum* bIV	
	6-instar	7-instar	6-instar	7-instar
Egg	19.1 ± 1.05	19.4 ± 0.21	18.6 ± 1.05	18.4 ± 0.68
Pupae (males)	27.4 ± 2.68	27.1 ± 0.63	28.0 ± 1.05	27.8 ± 0.96
Pupae (females)	26.5 ± 0.63	27.2 ± 0.71	27.5 ± 1.21	27.7 ± 0.50
Adult (males)	26.9 ± 1.05	26.7 ± 1.13	26.8 ± 0.21	25.2 ± 0.13
Adult (females)	26.2 ± 0.84	25.9 ± 0.50	24.2 ± 0.96	23.6 ± 0.71

On the basis of results mentioned above the reproductive effort was estimated using three different measures: the progeny-to-female ratios were calculated on the basis of: (1) lipid contents in eggs laid daily by a female to live weight of reproducing female, (2) lipid content in daily eggs production to lipid content in a pupal female, and (3) lipid contents in daily egg production to lipid content in adult female (Table 3). Apart from the ratio based on lipid content and body weight, the reproductive effort was also estimated as based on energetic equivalent. Estimates resulted in two indices – when energy in daily egg deposit was related: (4) to that in pupal female and (5) to adult female (Table 4).

Table 3. Calculation of reproductive effort indices in *Tribolium* (based on lipid content)

Elements used for calculation	*T. castaneum* cI		*T. confusum* bIV	
	6-instar	7-instar	6-instar	7-instar
1. Lipids in egg (μg)	1.61	1.97	3.28	3.9
2. Lipids in daily egg production by female (μg)	32.20	33.40	38.11	34.95
3. Weight of female (μg)	1950	2300	2580	2830
4. Lipids in pupal female (μg)	346.3	440.2	485.2	637.8
5. Reproductive effort (a) 2 : 3	**1.7**	**1.5**	**1.5**	**1.2**
6. Reproductive effort (b) 2 : 4	**9.3**	**7.6**	**7.9**	**5.5**
7. Dry weight of adult female (μg)	799.5	943.3	1118.2	1227.6
8. Lipids in adult female (μg)	168.5	183.8	219.2	236.4
9. Reproductive effort (c) 2 : 8	**19.1**	**18.2**	**17.4**	**14.8**

Table 4. Calculation of reproductive effort indices in *Tribolium* (based on energetic value)

Elements used for calculation	*T. castaneum* cI		*T. confusum* bIV	
	6-instar	7-instar	6-instar	7-instar
1. Energy in egg (J)	0.41	0.38	0.65	0.64
2. Energy in daily eggs production by female (J)	8.19	6.54	7.52	6.20
3. Dry weight of pupal female (μg)	913	1116	1243	1365
4. Energy in pupal female (J)	24.2	30.3	34.2	37.8
5. Reproductive effort (d) 2 : 4	**33.8**	**21.6**	**22.0**	**16.4**
6. Dry weight of adult female (μg)	799.5	943.3	1118.2	1227.6
7. Energy in adult female (J)	20.9	24.5	27.0	28.9
8. Reproductive effort (e) 2 : 7	**39.2**	**26.7**	**27.8**	**21.4**

All ratios are higher in *T. castaneum* than in *T. confusum*, pointing to a higher reproductive effort in the former species. If we compare 6- and 7-instar groups within each species, we come to a conclusion that 6-instar group is characterised by higher reproductive effort as evidenced by higher value of all these indices.

A percentage share of energetic resources spent for eclosion and maturation in the total amount accumulated in pupae is presented in Table 5. In all cases 7-instar individuals use for reaching maturity larger part of accumulated resources than 6-instar ones. *T. confusum* needs for that purpose larger fraction of resources than *T. castaneum* (lipid ratio in males is the only exception).

Table 5. Share of energetic resources used for eclosion and maturation of the amount stored in pupae of males and females of *Tribolium* (lipids or energy units)

Sex	*T. castaneum* cI		*T. confusum* bIV	
	6-instar	7-instar	6-instar	7-instar
		in lipids units (%)		
Males	47.5	57.6	44.8	54.9
Females	41.2	58.4	54.8	62.9
		in energy units (%)		
Males	14.4	20.0	23.0	25.6
Females	13.6	19.3	20.9	23.5

4. DISCUSSION

Lipid content is highest in pupal stage in both species. It is in accordance with results obtained earlier (Klekowski et al. 1967) for *Tribolium castaneum.* In the present paper it is clear that lipid content in eggs and pupal stages of 7-instar group is higher than that of 6-instar group of the two species. It is another trait that underlines the intrapopulation differentiation in investigated populations. Such traits as developmental time, number of moults, maximum weight in larval stages and fecundity, were investigated earlier in these species (Prus 1976, Bijok 1986, T. Prus and M. Prus 1987, Prus et al. 1988).

It is interesting that in males of *T. castaneum* almost identical content of lipids was observed in 6- and 7-instar pupae, whereas in *T. confusum* it was higher in 7-instar than in 6-instar pupae. In adult males, the situation was similar in *T. castaneum*, and reversed in *T. confusum*. In females, 7-instar pupae have always higher lipid content whereas in adult females the opposite situation was observed.

In energetics value such differences are not univocal, although the trends between instar groups and two species are maintained. The differences between females in pupal and adult stages were similar. Values both in lipid and energy contents were lower in adult females than in pupal females, which can be attributed to the reproduction process. The energy expenditure is very intensive during the first month of adult live (Prus et al. 1988).

The loss of energy or of lipid material can be a good measure of how high is the cost of maturation and eclosion in *Tribolium* beetles. If one relates the amount of energy used for these processes to all energy or material stored in pupae, one can evaluate the cost of maturation in different substrains of the species examined (Table 5).

In terms of lipid content the maturation cost is higher in 7-instar groups (over 55%) which holds for both sexes in the two species. The lowest relative expenditure of energy for maturing and eclosion is observed in 6-instar females of *T. castaneum* – 41.2%. These results are in accordance with results obtained by Clarke (1977) for benthic prawn, *Chorismus antarcticus* (Pfeffer). In the latter species, females synthetise for the gonad development an average value of 50% of their own lipid content. The same proportions were observed when considering the cost of maturation and reproduction in terms of energy content, however the percentages were much lower and ranged from 14% in 6-instar females of *T. castaneum* to 25% in 7-instar males of *T. confusum*.

Reproductive effort expressed as ratio of lipid content in eggs produced daily by a female to its fresh weight depicts real situation in examined strains, since the weights of females were assessed on 72nd day of life and about 42nd day after mating. According to Clarke (1979), when reproductive effort is expressed in terms of fresh weight biomass only then the results are comparable. If reproductive effort is expressed in terms of lipid or energetic content, considerations such as nitrogen availability for yolk protein, availability of amino acids and fatty acids or phosphorus are ignored.

Stearns (1992) considers that "the ratio of reproductive biomass to the mass of the parent, or of calories to calories are static measures that do not represent the proportion of energy flowing through the organism that is devoted to reproduction". His opinion is strengthened by quoting Hirshfield and Tinkle (1975). Nevertheless to avoid naive comparisons the approach in the present paper involves daily ratio of energy allocated to reproduction. Since *Tribolium* shows in laboratory condition a continuous reproduction this makes an equal time basis for comparison, no matter how long a female can be in reproductive state in each species or phenotypic group. Hence, evaluation of reproductive effort on diurnal rate renders dynamic approach.

The opinion presented by Tinkle and Hardley (1975) is that estimation of reproductive effort as based on the total energy budget is the best mean of characterising the compared species or strains. However, the energy budget assessments differ so much one from another, depending on: developmental cycle, type of feeding, reproduction and behaviour, that even estimates based on total energy budgets can be illusive and not valid for comparison of more distant taxonomic units. For example, in *Tribolium* data on total reproduction during the whole life span are still missing or it is difficult to measure them. Such are also budget parameters. It seems, however, that for future studies in *Tribolium* we should choose only instantaneous daily energy budgets of a reproducing female.

5. SUMMARY

Lipid content and energetic value in eggs, pupal stage and adults in two species of *Tribolium* and within discerned groups were investigated.

These values in eggs and pupal stages were higher in 7-instar group than those in 6-instar group. In adult males the lipid content and energetic value were higher than that in females, such

differences are visible in *T. confusum* (Tables 1, 2). The reproductive effort was calculated as based on proportion between energy or lipids stored in the parental female and in its progeny. This effort is highest in 6-instar group of *T. castaneum*, lowest –in 7-instar group of *T. confusum* (Tables 3, 4).

The shares of energetic resources used by animals for eclosion and maturation for both discerned groups in the two species were calculated (Table 5).

Both lipid content and reproductive effort besides other demographic traits have confirmed the occurrence of intrapopulation differentiation in these species.

6. POLISH SUMMARY

U dwóch gatunków *Tribolium* i u dwu wyróżnionych w ich populacjach grup określono zawartość lipidów i wartość energetyczną jaj, poczwarek i dorosłych osobników.

Stwierdzono wyższą zawartość lipidów i wyższą wartość energetyczną jaj i poczwarek u grupy 7-stadialnej niż u 6-stadialnej. U dorosłych samców określone wartości są wyższe niż u samic, zwłaszcza w grupie 7-stadialnej u *T. confusum* (tab. 1, 2). Na podstawie otrzymanych wyników obliczono wysiłek reprodukcyjny (tab. 3, 4). Najwyższą wartość tego parametru stwierdzono w grupie 6-stadialnej *T. castaneum*, a najniższą – u grupy 7-stadialnej *T. castaneum*. Udział zasobów energetycznych zużytych przez zwierzęta na przeobrażenie i dojrzewanie u badanych grup u obu gatunków wynosi średnio 55% (tab. 5). Stwierdzono, że ocena zawartości lipidów i wysiłku reprodukcyjnego, obok innych wcześniej zbadanych parametrów demograficznych, potwierdza istnienie zróżnicowania wewnatrzpopulacyjnego u tych gatunków.

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