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Incubation, hatching and yolk utilization in the eggs of the orb weaving spider *Cyrtophora cicatrosa* (Araneae; Araneidae)

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Abstract. *Cyrtophora cicatrosa* Stoliczka was reared from hatching to death at 4 different temperatures i.e. 22, 27, 32 and 37° C. A significant correlation is obtained between initial water content and hatchability of the eggs. During embryonic development energy density and fat decreased but the water content increased. Fat was the main energy source for embryonic development. Of 0.34 gcal energy present in an egg, 29% is utilized on embryonic metabolism, 21% on postemergent activities.

Keywords. Incubation; yolk utilization; hatchability; water content of eggs; *Cyrtophora cicatrosa.*

1. Introduction

The pattern of yolk utilization is known to reflect the physiological aspect of the embryo in relation to environment (Needham 1931). The spider egg must contain enough energy and nutrients to meet the metabolic cost of embryonic development, emergence, dispersal and other post-emergent activities (Anderson 1978). Aerial dispersal by ballooning may heavily tax yolk energy reserves of a spiderling (Valerio 1977). Subsequently the spiderling must be able to establish a web, before it can draw energy from food. Unlike the young ones of crustaceans and insects, a spiderling requires an additional fraction of yolk energy to meet the post-emergent activities. Hence, the pattern of yolk utilization in a spider is likely to exhibit adaptative strategy. Yet, no studies have been reported on the efficiency of yolk utilization in spiders. But there are numerous publications on this aspect for crustaceans (Pandian 1967, 1970a, b, 1972) and insects (Mathavan 1975). The present paper reports on the incubation, hatching and yolk utilization in the orb weaving spider *Cyrtophora cicatrosa* (Stoliczka).

2. Material and methods

C. cicatrosa inhabits commonly on the fence of *Euphorbia antiquorum* in Palni (10° 23′ N; 77° 31′ E) areas. It lays a string of egg sacs, which are hung like a beaded chain above the orb net. The oldest egg sac is at the top and the recent one at the bottom. This facilitated the collection and separation of eggs at different developmental stages from a string of known parentage.

The rate of development, was studied at 22, 27, 32 and 37° C at each temperature, sacs containing 150 eggs were used. The desired temperature was maintained to an accuracy of ± 1° C in a BOD incubator. To observe the effect of ration on incubation and hatching, eggs were obtained by rearing the spider fed *ad libitum*, which was equivalent to 100% ration; considering maximum food consumption of adult female, other rations such as 60, 30 or 15% were formulated and thus eggs were obtained by feeding the spiders with fixed quantity of prey. The eggs were maintained under even illumination for 12 hr/day. A cotton pad soaked in water provided uniform humidity (approximately 85% RH). Hatching was observed through stereo-dissection microscope.

For chemical and calorific analyses, the following developmental stages were chosen:

Stage I Undeveloped egg — soon after oviposition — nearly transparent — spherical with a diameter of 0.7 to 0.9 mm (age 1 day)

Stage II Mid embryonic period — heart beats — translucent — oval in shape (age 4-6 days)

Stage III Fully formed embryo — pear-shaped — cephalothorax, abdomen and appendages visible through chorion (age 9-11 days)

Stage IV Freshly hatched spiderling — with plenty of yolk in opisthosoma — not yet able to spin its web — moves its appendages continuously (age 12 days)

Stage V Young spiderling — yolk fully spent — active — able to spin its own dense snare — attained characteristic pigmentation by tanning of cuticle (age 16+2 days)

Water content of developing eggs and spiderlings was determined by weighing the test material before and after drying at 90° C to weight constancy. Organic substance was estimated subtracting ash content from dry matter. Ash was determined incinerating the test material before and after drying (about 40 mg dry substance) in a muffle furnace at 560° C for 6 hr and weighing the residue, as recommended by Paine (1964).

Fat content was estimated as the difference between the dry weight (40 mg) and fat-free dry weight of the test substance, determined after 8 hr extraction with petroleum ether in semi-micro soxhlet apparatus, according to the method detailed by the Association of Official Agricultural Chemists (1950).

The eggs and spiderlings were weighed in a single pan balance (Mechaniki model) to an accuracy of 10 μg. Calorific content of different developmental stages was determined in a semimicro bomb calorimeter (Parr Instrument Co.,) following the standard procedure described in Parr Instruction Manuals No. 128 and 130. When the sample was small (15 mg), a known quantity (25 mg) of benzoic acid was added to the sample and the combined calorific value was estimated.

3. Results

In *C. cicatrosa* development proceeds uninterrupted, *i.e.* diapause does not occur at the tested temperatures. Once eclosion is commenced, hatching of the simultaneously incubated eggs lasts for 2 days at temperatures 27° C and 6 days at 22° C. The incubation period is extended from 7 days at 37° C to 24 days at 22° C (table 1).

A number of factors influence hatchability of the incubated eggs. Age of the female appears to be an important factor; hatchability decreased from over 90% in the first 7 egg sacs to 40% in the 16th sac (table 2). Incidentally, the eggs oviposited by aged females contain less water. In spiders exposed to different temperatures, water content

Table 1. Effect of temperature and ration (at 27° C) on incubation and hatchability in *Cyrtophora cicatrosa.*

Temperature/ Ration	Incubation period (day)	Water (%)	Hatchability (%)
37° C	7 ± 1.1	27.1 ± 6.1	2 ± 0.3
32° C	9 ± 1.3	71.1 ± 6.8	82 ± 2.9
27° C	12 ± 1.7	65.6 ± 4.3	91 ± 3.7
22° C	24 ± 3.1	65.8 ± 5.1	75 ± 5.5
100%	12 ± 1.7	64.6 ± 4.5	90 ± 3.6
60%	12 ± 1.7	69.1 ± 4.8	74 ± 5.2
30%	12 ± 1.7	62.4 ± 4.1	67 ± 3.7
15%	12 ± 1.7	50.0 ± 3.6	55 ± 4.1

Each value (mean ± SD) represents the mean obtained from 21-36 egg sacs oviposited by *ad libitum* fed females. Water content of the eggs was determined soon after oviposition.

Table 2. Effect of female age on egg hatchability.

Age of female (day)	Egg sac number	Water content (%)	Hatchability (%)
96	1	65 ± 4.3	93 ± 2.1
101	2	66 ± 5.1	96 ± 1.7
107	3	68 ± 3.6	96 ± 2.2
112	4	69 ± 3.6	92 ± 3.3
117	5	71 ± 2.5	94 ± 2.7
121	6	68 ± 3.1	91 ± 4.1
126	7	68 ± 2.9	91 ± 5.3
132	8	59 ± 6.1	88 ± 4.6
139	9	66 ± 4.2	81 ± 6.7
146	10	69 ± 2.4	67 ± 7.6
155	11	69 ± 1.9	69 ± 5.2
163	12	60 ± 4.7	65 ± 8.1
172	13	63 ± 3.8	46 ± 10.2
181	14	53 ± 4.6	40 ± 9.2
190	15	55 ± 5.3	42 ± 16.2
200	16	48 ± 6.8	40 ± 14.6

Each value (mean ± SD) represents the mean obtained from 7-12 egg sacs oviposited by *C. cicatrosa* fed *ad libitum* at 27° C

of the eggs, another important factor, progressively decreased from 65% in the eggs of first few sacs to less than 50% in those contained in the last few egg sacs. Temperature also affects hatchability, which was around 80% in eggs incubated at 22, 27 or 32° C; at 37° C it was 2% only. Similarly, the eggs oviposited by females exposed to diminishing rations contain less water and correspondingly, the hatchability of these eggs is also reduced. On the whole, a significant positive relation ($P < 001$) became apparent between hatchability and water content of eggs oviposited under different rearing conditions; the correlation coefficient obtained ($r = 0.779$) for this relation indicated that water content significantly affected hatchability.

Spiderlings hatched throughout the day but the peak hatchability occurred at midnight. Considering the total eggs incubated, the maximum number was hatched (40%) between midnight and 0300 hrs. A freshly hatched spiderling is completely

Table 3. Changes in chemical composition and calorific content of developing eggs/spiderlings in *C. cicatrosa*

Developmental stage	Water content	Fat	Ash	Protein	Energy
I	69 ± 8.6	40 ± 1.1	1.3 ± 0.06	58.7	6440 ± 193
II	74 ± 3.6	37 ± 0.7	1.3 ± 0.05	61.7	5995 ± 215
III	72 ± 3.6	32 ± 1.3	1.4 ± 0.06	66.6	5810 ± 189
IV	72 ± 2.9	30 ± 1.2	1.4 ± 0.07	68.6	5751 ± 196
V	77 ± 3.0	29 ± 1.0	1.4 ± 0.08	69.6	5325 ± 213

Each value (mean ± SD) represents the mean of 3-8 estimations: energy in gcal/g dry weight: others in %. Water content is given as a percentage of live weight and the values for fat, ash and protein are given as percentage of dry weight and energy in cal/g dry substance; each value (mean ± SD) represents the mean 4 to 40 estimates.

Table 4. Average changes of wet weight and dry weight of a single egg/spiderling of *C. cicatrosa* in different developmental stages at 27° C.

Developmental stage	Number of animals	Total No. of eggs/ spiderling counted	Mean live weight (μg/egg or spiderling)	Coefficient of variation
I	27	4150	168 ± 13.2	7.9
II	25	4250	191 ± 6.4	3.4
III	25	3950	195 ± 24.8	12.7
IV	22	3750	152 ± 13.5	8.9
V	36	5450	141 ± 17.3	12.3

devoid of any pigmentation and nearly transparent. It is quiescent, but begins to move its appendages steadily and continuously. These movements may help circulation of the blood. The spinning glands are not yet fully formed. Mouth parts are still incomplete; plenty of yolk material is retained in opisthosoma.

As the development proceeds from stage I to stage V, water content increased from 69 to 77% (table 3). During the corresponding period, protein also increased from 59 to 70%; ash content does not appreciably change, but fat decreased from 40 to 29%, which is also reflected in the depletion energy density from 6440 to 5325 gcal/g dry weight. This indicates that fat is utilized as the major energy source during embryonic development.

3.1 *Changes in live and dry weights*

Live weight of a single egg steadily increased from 168 μg in stage I to 195 μg in stage III but subsequently decreased to 141 μg in a spiderling (table 4). With advancing development, more than one-third of dry weight (20 μg) of yolk substance was used for embryonic metabolism.

3.2 *Efficiency of yolk utilization*

From the values reported in tables 3 and 4, average changes in chemical composition and (table 5) calorific content of a single egg/spiderling were calculated. Dry matter, organic substance, ash content and protein showed slight increase in stage III (prior to hatching) and then decreased progressively, as the development advanced.

Table 5. Chemical composition and calorific content of egg/spiderling in different developmental stages (weight in μg; energy in gcal)

Developmental stage	Water	Dry matter	Organic substance	Fat	Ash	Protein	Energy
I	115.6	52.2	51.51	21.07	0.69	30.42	0.34
II	141.8	49.3	48.66	18.11	0.64	30.54	0.30
III	140.7	53.7	52.96	17.30	0.74	35.44	0.31
IV	109.6	41.7	41.22	12.56	0.58	28.64	0.24
V	108.5	32.4	31.95	9.44	0.45	22.63	0.17

The yolk utilization efficiency is a ratio of 'body formed/body formed + yolk used for metabolism'. In *C. cicatrosa*, the efficiency values were 62, 62, 50, 74 and 45% for dry weight, organic substance, calorific content, protein and fat respectively. The differences in the values indicate the efficiency with which the different substances of the yolk were utilized.

During development (stages I to IV), *C. cicatrosa* spent about 0.1 gcal on metabolic process; of this, the oxidation of protein contributed 10%, while fat oxidation supplied 89% energy (table 5).

3.3 *Water metabolism*

Water content increased 166μg in stage I to 141μg in stage III and thereafter, it gradually decreased to 108μg (stage V). Increases in water content in II and III stages may be contributed through the accumulation of metabolic water. The amount of water absorbed from the atmosphere through chorion is also included in this quantity. Decrease in water content in stage IV is due to water loss to the environment on hatching.

4. Discussion

A statistically significant correlation obtained between initial water content and hatchability of *C. cicatrosa* egg is a very significant observation. Eggs containing less than 50% water do not hatch readily, but those containing less than the critical minimum of 30% never hatch. At 37° C, the incubated eggs contained about 30% water and did not hatch at all. Usually the eggs oviposited by (i) aged females, (ii) females receiving 30% ration and (iii) females reared under the elevated temperatures (> 32° C) contained less than 60% water. Failure to hatch and egg mortality appears to be one of the mechanisms, by which population size is regulated by extreme environmental factors such as prey scarcity and elevated temperature.

The fact that lipid serves as a mjor energy source during embryonic metabolism of the spider *C. cicatrosa* confirms the conclusion made for terrestrial animals in general by Needham (1931) and also confirms the results obtained for certain aquatic crustaceans (Pandian 1967; Katre 1977; Ponnuchamy *et al* 1979). The period between the hatching of the spiderling and its successful establishment involves aerial dispersion by ballooning and heavy expenditure of reserve yolk energy (Schaefer 1978; Anderson 1978). To support the metabolic requirements of spiderling during this phase, a large quantum of energy is retained (70%) at the time of hatching in *C. cicatrosa* for these post-emergent activities. Of the total energy (0.34 gcal) 29% of egg energy is utilized on embryonic and 21% on post embryonic metabolism; the remaining (50%) goes to the constitution of the spiderling.

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