Embryonic Development and Diapause Stage in *Panonychus* Mites (Acari: Tetranychidae)

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Embryonic development was studied to elucidate the diapause stage of four spider mite species belonging to the genus *Panonychus*. Liquid paraffin proved to be useful for continuous observation of living embryos. Embryonic development was divided into 18 stages, which were illustrated and described. The initial phase of embryonic development was characterized by two total and equal divisions. This was followed a superficial cleavage. Embryonic development of diapause eggs was suppressed at the late blastula stage shortly before the appendage formation.

Key words: Acari, *Panonychus* spp., embryonic development, diapause, cleavage

INTRODUCTION

Although developmental arrest of diapause eggs can take place in almost any stage among different species, the stage of embryogenesis at which diapause occurs is species-specific in most cases (BECK, 1980; DANKS, 1987). Eggs of the silkworm, *Bombyx mori* L., for example, enter diapause at the pyriform stage (UMEYA, 1946), while those of the chrysomelid beetle, *Atrachya menetriesi* FALDERMANN, do so at the appendage formation stage (MIYA, 1965; ANDO, 1978). Diapause occurs at the pharate larval stage in the gypsy moth, *Lymantria dispar* L. (MASAKI, 1956). However, some species, such as the grasshopper, *Austroicetes cruciata* SAUSS. (UVAROV, 1966) and the cricket, *Allonemobius fasciatus* DEGEER (TANAKA, 1984), show different diapause stages, depending on the temperatures to which the eggs are exposed.

In spider mites, embryonic development of diapause eggs has received little attention except in the case of the European red mite, *Panonychus ulmi* (KOCH). Diapause occurs at the blastoderm formation stage in this species (LEES, 1955). For non-diapause eggs, embryonic development has been examined by several researchers. For example, development of the two-spotted spider mite, *Tetranychus urticae* KOCH, and the lime spider mite, *Eotetranychus tiliarium* (HERMANN), is characterized by the occurrence of total division and the following superficial cleavages (DITTRICH, 1965, 1968, 1969; JEPSON et al., 1975; CROOKER, 1985). Similar observations have been reported for *P. ulmi* (GASSER, 1951) and the citrus red mite, *P. citri* (MCGREGOR) (FUKUDA and SHINKAJI, 1954). However, embryonic development of spider mites still remains poorly studied.

The purpose of this study was to determine the diapause stage of embryos in the genus *Panonychus*. For this purpose, we first examined the normal course of embryonic

development for the non-diapause eggs, and then observed diapause eggs in order to determine the stage at which diapause occurred.

MATERIALS AND METHODS

Mites. Four species of the genus *Panonychus* were used: *P. bambusicola* EHARA et GOTOH was collected from the dwarf bamboo, *Sasamorphia borealis* (HACKEL), in Ibaraki; *P. thelytokus* EHARA et GOTOH from the elm, *Ulmus davidiana* (REHD.), in Hokkaido; *P. ulmi* (KOCH) from the apple, *Malus pumila* MILL., in Hokkaido; and *P. mori* YOKOYAMA from the Japanese pear, *Pyrus serotina* REHD., in Chiba. Embryonic development was observed for both non-diapause and diapause eggs in the first three species, and for only diapause eggs in *P. mori*. Laboratory stocks were maintained on leaf discs of the appropriate host plants on water-soaked cotton in Petri dishes (9 cm diam.) at $25 \pm 1^\circ\text{C}$, 60–70% R.H. and 16L–8D.

Embryonic development. Liquid paraffin provides a superior buffer for continuous observation of the living embryos in spiders (YOSHIKURA, 1987), but had not been tested on spider mites. We therefore compared the hatchability and the duration of the egg stage between paraffin-treated and paraffin-free eggs under continuous illumination and $24\text{--}25^\circ\text{C}$. Eggs laid within 2 h were placed on a cavity slide filled with liquid paraffin (paraffin-treated eggs) or on a cavity slide without it (paraffin-free eggs). Paraffin-free eggs were observed every 2 h during the first 10 days, while paraffin-treated eggs were checked only for hatchability on the 10th day of deposition.

To obtain non-diapause eggs, adult females were kept at 16L–8D and 25°C (GOTOH, 1987; EHARA and GOTOH, 1992). Eggs obtained within 15 min after deposition were placed on a cavity slide with a droplet of liquid paraffin, and then a coverslip was put on it. During the first 18 h the eggs were observed every 15 min, and thereafter at 2-h intervals until almost all eggs hatched or stopped their development. Observations were made under a compound microscope in a room of continuous illumination and $24\text{--}25^\circ\text{C}$. The eggs on the slide were systematically numbered, and the developmental stages of embryos were individually recorded in order to determine the normal course of embryonic development.

To obtain diapause eggs, 0- to 2-day-old non-diapause eggs were kept at a 10L–14D photoperiod and $18 \pm 1^\circ\text{C}$ until they hatched and reached adulthood (GOTOH, 1989). Diapause eggs laid on a leaf disc by newly emerged females were moved onto a cavity slide within 2 h after oviposition and observed every 2 h during the first 3 days and then at 6-h intervals for the 4th–11th days after oviposition. This observation was made only on *P. bambusicola*. For the other 3 species, the embryonic development was examined every 6 h during the first 11 days to determine the diapause stage.

RESULTS AND DISCUSSION

Influence of liquid paraffin on embryonic development

Hatchability of paraffin-treated eggs was similar to or slightly lower than that of paraffin-free eggs in the three species tested (Table 1), indicating that liquid paraffin had no deleterious effect on embryonic development.

The hatchability of paraffin-free eggs was significantly lower than that of eggs maintained on a leaf. In *P. bambusicola*, 114 out of 126 eggs (90.5%) hatched on a leaf

Table 1. Hatchability and development of paraffin-treated and paraffin-free non-diapause eggs in three species of *Panonychus* at 25°C

Species	Egg condition	Hatchability (%)	N	χ^2 -test ^a	Egg period in h mean $\pm$ S.D. (range)	N	t-test ^a
<i>P. bambusicola</i>	free	53.6	209	4.05*	146.3 $\pm$ 10.60 (132 – 182)	112	0.61 ^{ns}
	treated	40.7	86		147.7 $\pm$ 12.81 (130 – 184)	29	
<i>P. thelytokus</i>	free	74.2	62	3.90*	164.1 $\pm$ 11.99 (138 – 184)	30	1.11 ^{ns}
	treated	56.3	48		169.4 $\pm$ 20.65 (138 – 210)	16	
<i>P. ulmi</i>	free	54.9	51	3.43 ^{ns}	159.9 $\pm$ 12.96 (138 – 190)	28	2.08*
	treated	37.3	59		169.2 $\pm$ 16.81 (134 – 200)	17	

^a Free vs. treated. * $p < 0.05$; ^{ns} not significant ($p > 0.05$).

($\chi^2=48.7$, $p < 0.001$). This difference may be due to the influence of handling: a brush was used to remove eggs from the leaf surface. Furthermore, hatchability in the paraffin-treated eggs was lower than that in the paraffin-free eggs in *P. bambusicola* and *P. thelytokus* (Table 1). It is possible that the volume of air in the egg was slightly low for hatching. YOSHIKURA (1987) has reported that liquid paraffin is harmless to embryonic development of spiders shortly before hatching, but hatching rarely occurs because of a shortage of air reaching the egg.

Duration of the egg stage in *P. bambusicola* and *P. thelytokus* was almost the same in the paraffin-treated and paraffin-free eggs, but in *P. ulmi* the treated eggs took about 10 h longer to develop than did the paraffin-free eggs (Table 1). The paraffin-treated eggs of *P. ulmi* spent about 28 h at the pharate-larva stage (Table 4), while the paraffin-free eggs spent only 12.7 ± 8.2 h ($N=43$). Thus, liquid paraffin is useful for observation of living embryo of spider mites.

Embryonic development of non-diapause eggs

Embryonic development in insects and mites has been described in different ways (e.g., MIYA, 1965; DITTRICH, 1968; YOSHIKURA, 1987; HUTU, 1991). In the present study, embryonic development was divided into 18 stages for convenience in the three species observed, although the durations of different stages varied among species (Tables 2, 3 and 4). Figure 1 illustrates the 18 developmental stages for *P. bambusicola* as an example.

At about 25°C, cell division occurred at about 1-h intervals before the 128-cell stage was reached. DITTRICH (1968) reported that in *T. urticae* 1,024 cells were seen in the egg after the tenth division. However, we could not distinguish more than 100 blastomeres. The three divisions from the 8th to 10th, therefore, were regarded as the morula stage.

The characteristics and durations of different stages were very similar among the three species examined, except that *P. thelytokus* and *P. ulmi* took longer to hatch after the eye-spot stage than *P. bambusicola* (Tables 2, 3 and 4).

The normal course of embryonic development was basically similar to the results

Table 2. Time and duration of different embryonic stages of non-diapause eggs in *P. bambusicola* at 24–25°C and continuous illumination^a

Developmental stage	Time of the beginning of stage (hours after deposition)	<i>N</i>	Duration (h)	<i>N</i>
A. Yolk	0	35	1.7±0.22 (1.25 – 2.00) ^b	35
B. One-cell	1.7±0.22 (1.25 – 2.00)	35	1.1±0.26 (0.75 – 1.75)	35
C. Two-cell	2.8±0.22 (2.50 – 3.25)	35	1.0±0.19 (0.75 – 1.50)	35
D. Four-cell	3.8±0.27 (3.25 – 4.50)	35	0.9±0.17 (0.50 – 1.25)	35
E. Eight-cell	4.7±0.23 (3.75 – 5.25)	35	0.8±0.16 (0.50 – 1.00)	35
F. 16-cell	5.5±0.29 (5.00 – 6.25)	35	0.9±0.14 (0.75 – 1.25)	35
G. 32-cell	6.4±0.30 (6.00 – 7.00)	35	1.0±0.16 (0.75 – 1.25)	35
H. 64-cell	7.4±0.36 (6.75 – 8.00)	35	1.0±0.16 (0.75 – 1.25)	35
I. 128-cell	8.4±0.43 (7.50 – 9.25)	35	1.1±0.22 (0.50 – 1.50)	35
J. Morula	9.5±0.51 (8.25 – 10.50)	35	5.7±0.76 (4.25 – 7.00)	35
K. Blastula I	15.2±0.73 (14.00 – 16.75)	35	4.5±1.33 (2 – 8)	33
L. Blastula II	19.0±1.91 (16 – 24)	33	3.8±2.28 (2 – 14)	33
M. Blastula III	22.4±2.02 (20 – 28)	33	12.6±3.17 (8 – 18)	33
N. Appendage formation I	35.4±2.50 (32 – 44)	34	22.8±4.52 (16 – 34)	34
O. Appendage formation II	58.2±5.19 (50 – 68)	34	39.7±11.91 (2 – 62)	34
P. Eye-spot	99.3±14.32 (58 – 126)	34	47.3±9.16 (14 – 64)	30
Q. Pharate larva	141.4±12.64 (122 – 180)	29	6.3±5.37 (2 – 22)	29
R. Larva	147.7±12.81 (130 – 184)	29		

^a Mean±S.D. ^b Range.

of DITTRICH (1965, 1968, 1969), although more detailed information is presented in this study.

Total and equal divisions were followed by a superficial cleavage at the third division in all the species observed, as was the case with other spider mites, viz. *T. urticae*, *E. tiliarium* (DITTRICH, 1965), *P. ulmi* (GASSER, 1951) and *P. citri* (FUKUDA and SHINKAJI, 1954). Thus, this pattern of embryonic development appears to be common

Table 3. Time and duration of different embryonic stages of non-diapause eggs in *P. thelytokus* at 24–25°C and continuous illumination^a

Developmental stage	Time of the beginning of stage (hours after deposition)	<i>N</i>	Duration (h)	<i>N</i>
A. Yolk	0	44	2.0±0.29 (1.25–2.50) ^b	44
B. One-cell	2.0±0.29 (1.25–2.50)	44	0.9±0.28 (0.50–1.75)	44
C. Two-cell	2.9±0.23 (2.50–3.50)	44	0.9±0.17 (0.50–1.25)	44
D. Four-cell	3.9±0.30 (3.25–4.75)	44	0.8±0.16 (0.50–1.00)	44
E. Eight-cell	4.7±0.35 (4.00–5.75)	44	0.8±0.15 (0.50–1.25)	44
F. 16-cell	5.5±0.39 (4.75–6.75)	44	0.9±0.14 (0.50–1.00)	44
G. 32-cell	6.4±0.44 (5.50–7.75)	44	0.9±0.13 (0.75–1.25)	44
H. 64-cell	7.3±0.44 (6.50–8.50)	44	0.9±0.12 (0.75–1.00)	44
I. 128-cell	8.2±0.50 (7.25–9.50)	44	0.9±0.12 (0.75–1.00)	44
J. Morula	9.0±0.53 (8.00–10.50)	44	4.3±0.60 (3.25–5.50)	44
K. Blastula I	13.3±0.82 (12.00–15.25)	44	5.0±1.56 (2–10)	44
L. Blastula II	20.0±1.36 (18–22)	50	3.0±1.28 (2–6)	50
M. Blastula III	23.0±2.00 (18–30)	53	9.7±2.66 (6–20)	53
N. Appendage formation I	32.8±2.78 (28–44)	53	24.6±3.88 (18–38)	53
O. Appendage formation II	57.4±5.38 (48–78)	53	37.1±6.71 (28–58)	53
P. Eye-spot	94.5±11.33 (76–136)	53	68.4±12.48 (46–98)	53
Q. Pharate larva	159.1±15.39 (130–192)	53	15.1±9.85 (4–36)	16
R. Larva	169.4±20.65 (138–210)	16		

^a Mean±S.D. ^b Range.

in Tetranychidae. The shift from total to superficial cleavages is also known in a parasitic mite (AKIMOV and YASTREBTSOV, 1990), spiders (YOSHIKURA, 1987), collembolan and Thysanura (ANDO, 1991).

Diapause stage

About 54 h after deposition, diapause eggs of *P. bambusicola* contained an embryo

Table 4. Time and duration of different embryonic stages of non-diapause eggs in *P. ulmi* at 24–25°C and continuous illumination^a

Developmental stage	Time of the beginning of stage (hours after deposition)	N	Duration (h)	N
A. Yolk	0	64	1.6±0.27 (1.00–2.75) ^b	64
B. One-cell	1.6±0.27 (1.00–2.75)	64	1.0±0.23 (0.75–1.75)	64
C. Two-cell	2.7±0.25 (1.75–3.50)	64	1.0±0.14 (0.50–1.25)	64
D. Four-cell	3.6±0.24 (2.75–4.00)	64	0.8±0.14 (0.50–1.00)	64
E. Eight-cell	4.4±0.27 (3.50–4.75)	64	0.8±0.15 (0.50–1.25)	64
F. 16-cell	5.3±0.26 (4.25–5.75)	64	0.9±0.12 (0.75–1.25)	64
G. 32-cell	6.2±0.25 (5.25–6.50)	64	0.9±0.15 (0.75–1.25)	64
H. 64-cell	7.1±0.29 (6.25–7.50)	64	1.0±0.13 (0.75–1.25)	64
I. 128-cell	8.1±0.32 (7.00–8.50)	64	1.0±0.16 (0.75–0.16)	64
J. Morula	9.0±0.34 (8.00–9.75)	64	4.6±0.56 (3.50–5.75)	64
K. Blastula I	13.6±0.59 (12.50–15.25)	64	4.5±1.60 (2–10)	49
L. Blastula II	17.5±1.39 (14–22)	49	3.1±1.41 (2–8)	49
M. Blastula III	20.6±1.89 (18–28)	50	11.0±2.34 (4–20)	50
N. Appendage formation I	31.6±2.01 (28–42)	50	14.8±5.41 (4–34)	50
O. Appendage formation II	46.4±5.09 (40–66)	50	48.5±9.57 (32–72)	50
P. Eye-spot	94.9±9.52 (80–118)	50	61.1±15.26 (30–92)	50
Q. Pharate larva	151.3±14.73 (126–182)	59	27.8±11.22 (4–44)	17
R. Larva	169.2±16.81 (134–200)	17		

^a Mean±S.D. ^b Range.

at stage M (Blastula stage III). No further development occurred thereafter (Table 5). In the other three species examined, viz. *P. mori*, *P. thelytokus* and *P. ulmi*, development also ceased at stage M. This shows that these mites enter diapause late at the blastula stage. At 18°C, embryonic development took about twice as long as that at 25°C (Tables 2 and 5).

LEES (1955) reported that in the eggs of *P. ulmi* diapause occurred at the formation

Fig. 1. Illustration of different stages of the embryonic development in *P. bambusicola*. Yolk stage (A), uncleaved egg filled with yolk; One-cell stage (B), one blastomere (nucleus) visible in the center of uncleaved egg; Two-cell stage (C), two blastomeres of the same size are observed, and a cleavage plain can be seen between the two nuclei; Four-cell stage (D), four blastomeres occur after total and equal division; Eight-cell stage (E); 16-cell stage (F); 32-cell stage (G); 64-cell stage (H); Morula stage (I), 8th to 10th divisions are involved; Blastula stage I (K), a dark spot (=blastoderm) appears in the center; Blastula stage II (L), the dark spot begins to spread after reaching the peripheral region; Blastula stage III (=periblastula) (M), the dark area spreads over the entire surface and germ band formation begins; Appendage formation stage I (N), primordial appendages (=extremities) begin to be formed along the early germ band; Appendage formation stage II (O), embryo turns to the lateral at the angle of 90 degrees and appendages are elongated; Eye-spot stage (P), reddish eye-spots appear; Pharate-larva stage (Q), internal organization is completed and air-bubbles are formed between egg shell and embryo; Larval stage (R).

Table 5. Time and duration of different embryonic stages of diapause eggs in *P. bambusicola* at 18°C and continuous illumination^a

Developmental stage	Time of the beginning of stage (hours after deposition)	N	Duration (h)	N
A. Yolk	0	20	4.0±0.63 (2-6) ^b	20
B. One-cell	4.0±0.63 (2-6)	20	2.0±0.00 (2-2)	13
C. Two-cell	5.4±0.91 (4-6)	17	2.0±0.00 (2-2)	16
D. Four-cell	7.0±1.00 (6-8)	23	2.0±0.00 (2-2)	13
E. Eight-cell	8.7±0.96 (8-10)	14	2.0±0.00 (2-2)	10
F. 16-cell	10.1±0.77 (8-12)	20	2.1±0.46 (2-4)	18
G. 32-cell	12.0±0.85 (10-14)	22	2.3±0.70 (2-4)	21
H. 64-cell	14.3±1.22 (12-16)	23	2.0±0.00 (2-2)	20
I. 128-cell	16.0±1.07 (14-18)	21	2.0±0.00 (2-2)	21
J. Morula	18.0±1.00 (16-20)	24	13.0±2.24 (10-18)	24
K. Blastula I	31.0±2.16 (28-36)	24	11.8±2.58 (8-16)	24
L. Blastula II	42.8±3.91 (36-52)	24	11.3±5.35 (4-26)	24
M. Blastula III	54.1±5.76 (46-66)	24		

^a Mean±S.D. ^b Range.

of blastoderm, and CROOKER (1985) indicated that development of diapause eggs was suspended at an early embryonic stage. The present study also shows that the diapause stage was the late blastula stage. Thus, it may be common that embryonic development in diapause eggs is suppressed at the blastoderm stage in Tetranychidae. It will be necessary to clarify whether the diapause stage of spider mites is influenced by the temperature of incubation, as reported in the cricket, *A. fasciatus* (TANAKA, 1984).

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