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A simple preparation technique for transmission electron microscopic investigations of acarine eggs

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Abstract

The scarce knowledge on the embryonic development of mites is mainly due to difficulties in preparing the specimens. Transmission electron microscopic investigations, however, are crucial to clarify structural characteristics, which are best comprehended by the completest possible insight into the successive developmental steps. The technique recommended here, including the use of sodium hypochlorite, enables sufficient material to be gathered in a simple manner.

Zusammenfassung

Eine einfache Präparationstechnik für transmissionselektronenmikroskopische Untersuchungen an Milbeneiern – Der geringe Wissensstand über die Embryonalentwicklung der Milben ist vor allem auf präparatorische Schwierigkeiten zurückzuführen. Für die Aufklärung der strukturellen Verhältnisse sind aber transmissionselektronenmikroskopische Untersuchungen, insbesondere eine möglichst lückenlose Betrachtung der einzelnen Entwicklungsschritte, unerläßlich. Die hier vorgestellte Technik unter Verwendung von Natriumhypochlorit bietet die Möglichkeit, das Material auf einfache Weise in genügend großem Umfang zu gewinnen.

Introduction

The embryonic development of mites is still poorly known due to the lack of detailed and comprehensive investigations. Most embryological studies have focused on Ixodida and Mesostigmata. Most of the available information is based on light microscopic evidence. Apart from a few SEM studies (AESCHLIMANN & HESS 1984, WALZL 1988, YASTREBTSOV 1992, THOMAS & TELFORD 1999) only two TEM accounts are available (CASPERSON et al.1986, FAGOTTO et al.1988). For clarification of structural characteristics during embryonic development, however, TEM use is indispensable (FAGOTTO et al. 1988).

Technical problems are responsible for our poor knowledge on mite embryology. This holds true equally for investigations conducted on the light microscopic level and on the electron microscopic level. These problems are ascribable on the one hand to the small size and great yolk content of the eggs, but, on the other hand, mainly to the impermeability of the egg shell and vitelline membrane for aqueous fixatives (AESCHLIMANN & HESS 1984, YASTREBTSOV 1992). While new techniques have been developed to solve such problems on the SEM level (WALZL & WAITZBAUER 1980, WALZL 1993, THOMAS & TELFORD 1999), successful approaches at the TEM level were hitherto lacking. A simple technique for gathering a suitable quantity of specimens for TEM analysis is presented below.

The Problem

To circumvent problems related to permeability, CASPERSON et al. (1986) and FAGOTTO et al. (1988) pierced the eggs. This procedure, however, has its own limitations:

i. because of the high intraoval pressure, eggs often burst, even with the use of hypertonic fixatives;

ii. controlled piercing, in order not to destroy areas to be investigated, is often very difficult or even impossible;

iii. sufficient infiltration of the eggs is limited to the area of piercing, even with the use of low viscosity resins such as SPURR.

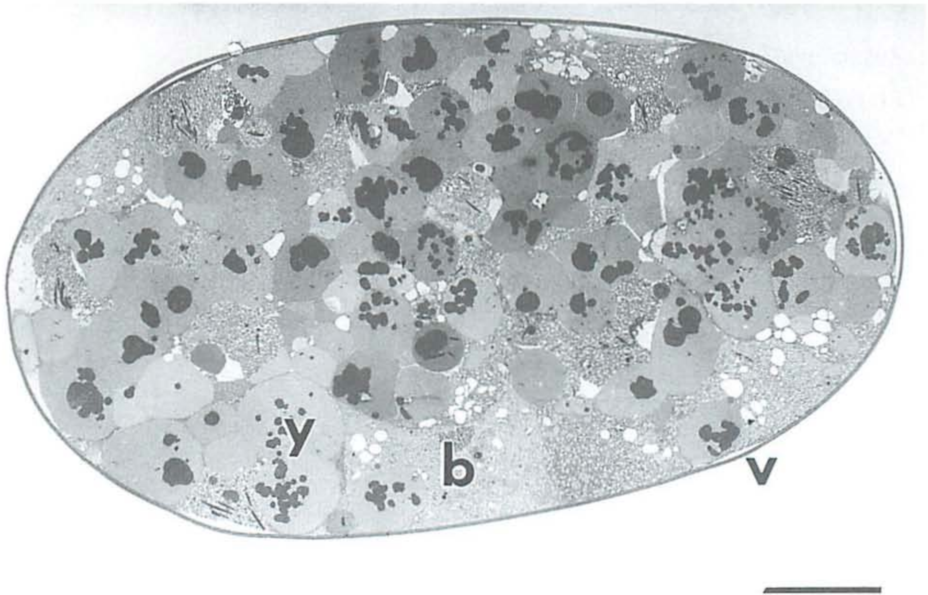

Fig. 1 Early blastoderm stage of *Sancassania berlesei* Michael, 1903. TEM overview, sagittal section. b = blastodermal cell, y = yolk platelet, v = vitelline membrane. Bar = 30 µm

The use of sodium hypochlorite as recommended in this study allows any desired quantity of specimens to be produced and makes piercing the eggs superfluous. These unpierced eggs, moreover, remain intact and the eggs are evenly infiltrated (Figs 1, 2).

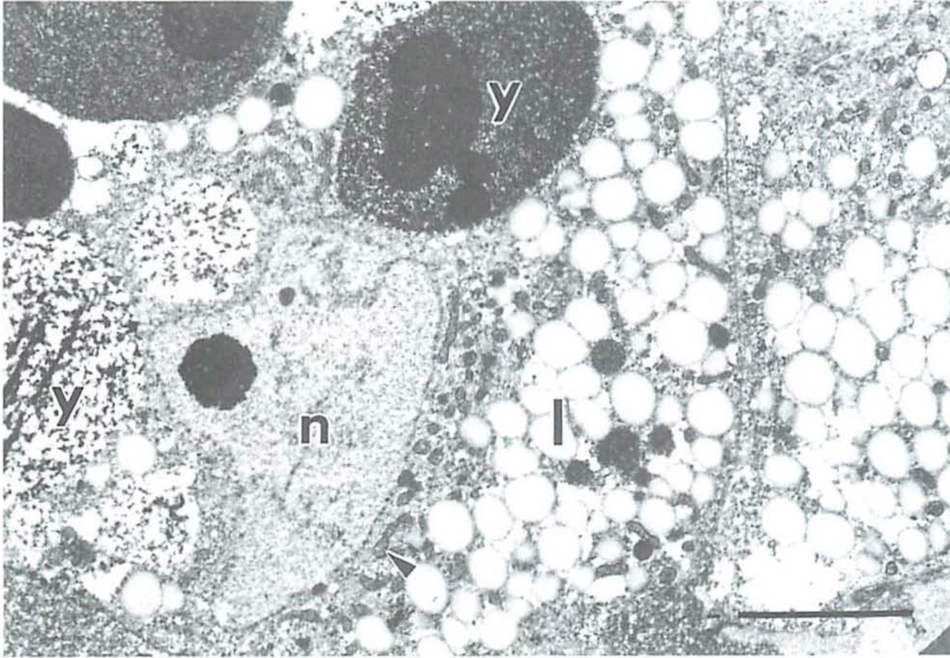

Fig. 2 *S. berlesei*. Same stage as fig. 1 at higher magnification. l = lipid droplet, n = nucleus of blastodermal cell, y = yolk platelet, arrowhead = mitochondrion. Bar = 3 µm

Technique

Special baskets are used in order to simultaneously process the desired quantity of eggs through all steps of our procedure. These consist of BEEM-capsules, the bottom of which has been replaced by a fine-meshed polyamide gauze (45 µm), attached with a fine-tipped soldering iron.

i. place for 120 s in 0.5 % sodium hypochlorite solution (a commercial hygienic cleaner, DanKlorix, is used);

ii. rinse 3 times in 0.1 M cacodylate buffer (4 % sucrose);

iii. primary fixation for 15 min in Karnovsky's fixative (4 % sucrose);

iv. 2 x 5 min in 0.1 M cacodylate buffer;

v. secondary fixation for 30 min in 2 % osmium tetroxide;

vi. 2 x 5 min in distilled water;

vii. 5 min in 50 %, 70 %, 80 % and 90 % ethanol;

viii. 2 x 5 min in 100 % ethanol;

ix. 30 min each in 100 % ethanol : resin (SPURR) 3:1, 2:1, 1:1, 1:2, 1:3;

x. 2 x 1 h in pure resin;

xi. polymerisation at 60 °C for 18 h.

To apply our procedure successfully, the following points are recommended. Exposure times to sodium hypochlorite less than 120 seconds result in insufficient infiltration. Exposure times longer than 120 seconds damage the eggs; this ranges from tissue destruction to bursting. Investigations of eggs of other mite taxa, however, may require modified exposure time to the sodium hypochlorite, due to possible structural differences in egg shell and vitelline membrane.

Shortening the procedure by omitting two or four infiltration steps (100 % ethanol : resin), as well as abbreviating the respective infiltration times do not yield satisfying results.

The addition of 4 % sucrose to the buffer solution and the fixative turned out to be advantageous for the structural preservation of tissues.

Discussion

Sodium hypochlorite (»bleach«) has hitherto not been used as a means of removing the shell of mite eggs in TEM investigations. The only recorded use of 5 % bleach is by THOMAS & TELFORD (1999), who investigated of the eggs of the oribatid *Archegozetes longisetosus* Aoki, 1965. These authors, however, used sodium hypochlorite in combination with a heptane-formaldehyde fixation and subsequent devitellinisation in a heptane-methanol solution. Such a procedure, applied to eggs of *Drosophila* ssp., is suitable for fluorescence and SEM studies; its use in TEM investigations, however, led to unsatisfactory results (WIESCHAUS & NÜSSLEIN-VOLHARD 1986).

The use of bleach in connection with standard fixation and infiltration procedures yields very satisfying results within acceptable time frames. This is especially crucial in embryological studies, which aim at demonstrating continuous series of developmental steps and require considerable quantities of material.

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