

## The structural basis for water exchange between the female cockroach (*Blattella germanica*) and her ootheca

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### Summary

Female German cockroaches usually carry their oothecae until they hatch. The success of embryogenesis may be dependent on the water-balance relationship between females and their developing oothecae. Oothecae detached from females early in embryogenesis often fail to develop, especially in low-humidity environments. Experiments reported here using tritiated water have confirmed the transport of a significant amount of water to the ootheca from the female during embryogenesis; 18% of the tritiated water injected into gravid females was recovered in their oothecae after 24 h. We describe a structural basis of water absorption by the oothecae. An area located on the proximal end of the ootheca (adjacent to the ‘escutcheon-shaped vaginal imprint’) contains small pores that penetrate the oothecal covering to access regions of the chorion lying beneath these pores. Experiments using microparabiotic chambers to examine transport of tritiated water and water-soluble materials

across the escutcheon region of the oothecal covering, along with dark-field birefringent microscopy of the chorion, support the hypothesis that this chorionic network is capable of conducting water throughout the interior of the ootheca. Furthermore, the structural arrangement and intimate association of the female vestibulum with the oothecal pore field contained in the region of the escutcheon-shaped vaginal imprint appear to provide an efficient conduit to the chorion. The overall structural relationship might be a means for maintaining water balance between females and their oothecae during embryonic development. Evidence presented here supports observations that this species represents an important link in the transition from oviparity to ovoviviparity by internalization of cockroach oothecae.

Key words: *Blattella germanica*, cockroach, water relations, oothecae, chorion.

### Introduction

Reproductive strategies among cockroaches are diverse and include oviparity, ovoviviparity and viviparity, as well as intermediate types resulting in various options for postparturition parental care (Nalepa and Bell, 1997; Roth, 1968; Roth and Willis, 1954, 1958). German cockroaches (*Blattella germanica*) are oviparous, and females typically carry their oothecae until they hatch. This longer-term association makes them functionally similar to ovoviparous species; as a result, they have been described as displaying ‘false’ ovoviviparity (Roth, 1989). Support for this designation is derived from observations that the proximal (attached) end of the ootheca is less sclerotized than other parts of the ootheca and is permeable to water, allowing for female participation in maintaining the water balance of the eggs (Roth and Willis, 1955a, 1958). Premature detachment of an ootheca from a female can result in death of the developing embryos (Barson and Renn, 1983; Roth and Willis, 1955c). Evolutionary trends from oviparity towards ovoviviparity include oothecal internalization within the female, a reduced oothecal covering, and close association of developing embryos with the female brood sac. Nalepa and Bell (1997) have noted that, except for

retraction of the ootheca into the body, female *B. germanica* exhibit all of the characteristics of cockroach ovoviviparity.

The mechanisms of oothecal formation, general structure (Wheeler, 1889; Ross, 1929; Wigglesworth and Beament, 1950; Lawson, 1951) and embryonic development (Tanaka, 1976) in cockroaches are available from earlier work. However, Hinton (1981) observed that there is a need for more-detailed structural information, including three-dimensional relationships of oothecal components. In this study, we have examined some morphological and physiological aspects of the association of *B. germanica* females and their oothecae in an attempt to provide new information on the nature of this association.

### Materials and methods

#### *Insect culture*

German cockroaches (*Blattella germanica* L.) used in this study were obtained from established Virginia Polytechnic Institute and State University cultures, held at approximately 20°C and ambient humidity in 41 glass battery jars and

provided with dog food and water *ad libitum*. Late-instar nymphs were removed from main cultures and held separately. Newly eclosed adults emerging from these groups were removed and divided into separate age groups. Mated females from these age-selected groups, carrying their initial ootheca, were used for the individual experiments reported here.

#### Radiolabel studies

##### Whole-body transport

Two experiments were conducted to examine water-distribution patterns between females and their oothecae. The females were either allowed access to water (W) *ad libitum* or deprived of water (W/O) for 24 h. 500 nl volumes of  $^3\text{H}_2\text{O}$  (specific activity  $1 \text{ mCi ml}^{-1}$ , New England Nuclear, Boston, MA, USA) were injected into abdomens of cold-immobilized ( $5^\circ\text{C}$ ) females carrying 11–20-day-old oothecae. This was accomplished by using a 0.25 ml glass syringe with a 32 gauge needle and a motor-driven microapplicator. After incubation at time intervals of 0.5 h, 2 h, 6 h or 24 h, the females were cold immobilized and their oothecae detached. The oothecae were rinsed with distilled water, blotted dry and placed on a glass microscopic slide mounted on a small block of dry ice. After the oothecae were frozen, a chilled razor blade was used to cut the oothecae into either equal quadrants (W) or sextants (W/O). The females and respective oothecal sections were placed in separate microcentrifuge tubes containing 500  $\mu\text{l}$  distilled water and were then subjected to a freeze ( $-70^\circ\text{C}$ )/thaw ( $24^\circ\text{C}$ ) regimen three times before homogenization with a

microcentrifuge pestle. The homogenates were centrifuged at  $1000g$  (5 min), and 250  $\mu\text{l}$  of the supernatant was placed in 8 ml Ecoscint scintillation fluid (National Diagnostics, Atlanta, GA, USA) and analyzed using a Beckman LS-3150 scintillation counter (Beckman Instruments, Inc., Irvine, CA, USA).

##### Permeability of the oothecal covering to water and water-soluble molecules

The relative permeability of radiolabeled water-soluble molecules across portions of the oothecal covering was determined using microparabiotic chambers (Fig. 1). These microparabiotic chambers were constructed from two pieces of glass tubing [ $2 \text{ cm length} \times 6 \text{ mm o.d./1.25 mm i.d.}$ ], each adapted with three tapered catch hooks that were spaced equidistantly around their circumferences on one end. In order to ensure a water-tight seal around the specimen, a 6 mm parafilm gasket with a 1 mm pore punched into the center was placed on the end opposite to the catch hooks of each chamber section. A  $2 \text{ mm}^2$  section of the oothecal covering, including the escutcheon-shaped vaginal imprint area, was then removed from the proximal end of the ootheca and placed between the two sections of the chamber. As the system was assembled, a larger glass tubing sleeve [ $2 \text{ cm length} \times 9 \text{ mm o.d./6 mm i.d.}$ ] was placed over the chamber ends, with the sample sandwiched between the parafilm gaskets. Three dental rubber bands were then attached to the catch hooks to hold each unit in place and ensure sufficient pressure to provide a good seal (see Fig. 1). 30  $\mu\text{l}$  of buffer ( $10.3 \text{ g l}^{-1} \text{ NaCl}$ ,  $1.46 \text{ g l}^{-1} \text{ KCl}$ ,  $0.36 \text{ g l}^{-1} \text{ NaHCO}_3$ ,  $0.21 \text{ g l}^{-1} \text{ NaH}_2\text{PO}_4$ ,  $0.36 \text{ g l}^{-1} \text{ NaHCO}_3$ ,  $0.21 \text{ g l}^{-1} \text{ NaH}_2\text{PO}_4$  and  $3 \text{ g l}^{-1} \text{ glucose}$ ; the pH was adjusted to 7.4 with  $1 \text{ mol l}^{-1} \text{ NaOH}$ , as described by Kurtti and Brooks, 1976) was delivered to the chamber end facing the interior side of the oothecal tissue, and 30  $\mu\text{l}$  of the Kurtti and Brooks buffer containing one of the radiolabeled materials listed below and fluorescein ( $50 \mu\text{g ml}^{-1}$ ) was delivered to the chamber end facing the exterior side of the oothecal tissue. At various time intervals (0 h, 1 h, 3 h, 6 h or 24 h), 1  $\mu\text{l}$  buffer was removed from the interior side of the chamber for radioassay, after which both exposed ends of the chamber were sealed with parafilm. The chambers were examined microscopically to ensure that air pockets had not formed between the tissue samples and the buffer solution. Samples removed from each end of the chamber at various time intervals were delivered to 20 ml scintillation vials containing Ecoscint scintillation fluid and were counted using routine radioassay techniques. The following radiochemicals were used:  $^3\text{H}_2\text{O}$ , specific activity  $1 \text{ mCi ml}^{-1} = 37 \text{ MBq ml}^{-1}$  (New England

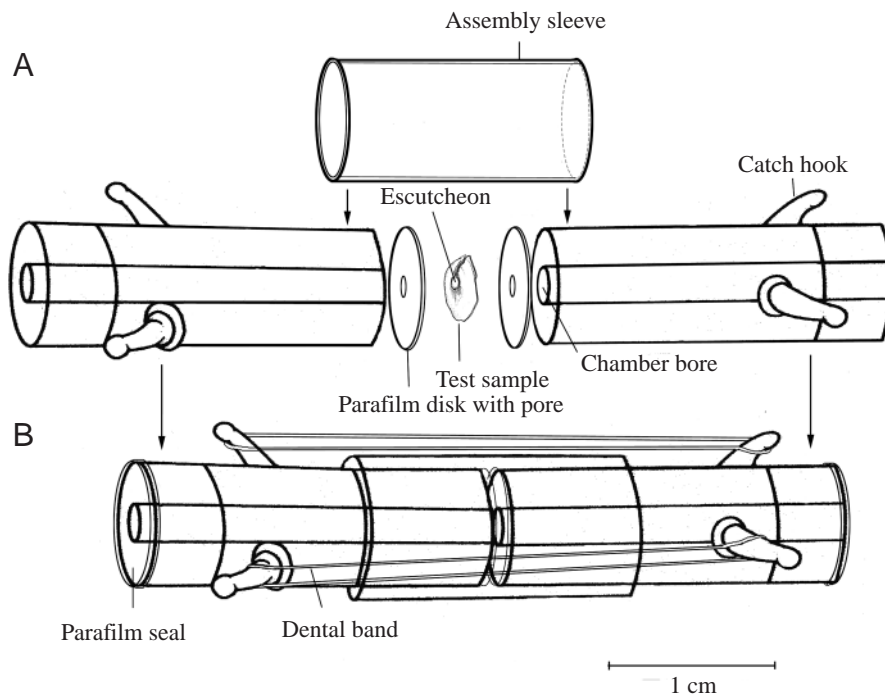


Fig. 1. Microparabiotic chamber constructed from glass tubing, designed to allow for measurement of water-soluble materials across small pieces of oothecal cuticle. (A) Exploded view of the chamber. (B) Assembled view of the chamber. See Materials and methods section and Fig. 4 for details. Scale bar, 1 cm.

microcentrifuge pestle. The homogenates were centrifuged at  $1000g$  (5 min), and 250  $\mu\text{l}$  of the supernatant was placed in 8 ml Ecoscint scintillation fluid (National Diagnostics, Atlanta, GA, USA) and analyzed using a Beckman LS-3150 scintillation counter (Beckman Instruments, Inc., Irvine, CA, USA).

Nuclear); [U-<sup>14</sup>C]glucose, specific activity 9.1 mCi mmol<sup>-1</sup>= 337 MBq mmol<sup>-1</sup> (ICN Radiochemicals, Irvine, CA, USA), [1-U-<sup>14</sup>C]L-leucine, specific activity 52 mCi mmol<sup>-1</sup>= 1.92 GBq mmol<sup>-1</sup> (ICN Radiochemicals); [U-<sup>14</sup>C]formate, specific activity 56 mCi mmol<sup>-1</sup>=2.07 GBq mmol<sup>-1</sup> (ICN Radiochemicals); [U-<sup>14</sup>C]glycine, specific activity 107 mCi mmol<sup>-1</sup>=3.96 GBq mmol<sup>-1</sup> (Amersham Searle, Piscataway, NJ, USA) and [<sup>14</sup>C]NaHCO<sub>3</sub>, specific activity 51 mCi mmol<sup>-1</sup>=1.89 GBq mmol<sup>-1</sup> (ICN Radiochemicals).

Microscopy

Scanning electron microscopy

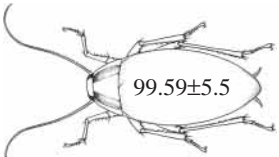
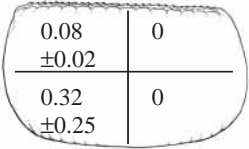
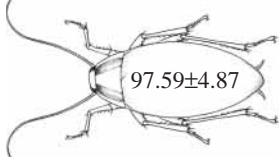
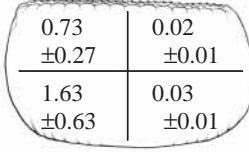
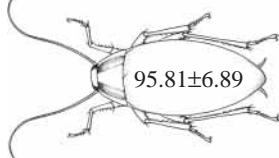
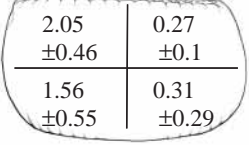
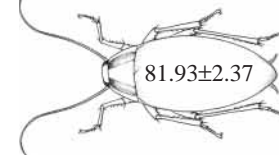
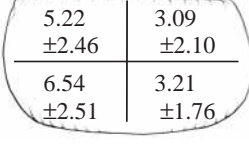
11–21-day-old oothecae were removed from females, frozen and separated into proximal and distal ends with a razor blade. The oothecal sections were lyophilized, mounted onto metal stubs with aluminum paint, and sputter-coated with colloidal carbon. The images were obtained using a Philips 505 Scanning Electron Microscope (SEM). In order to observe the internal surface of the oothecal covering, it was necessary to remove the chorion, which is closely associated with the

internal surface. This was done by treating 2–3 mm<sup>2</sup> pieces of the escutcheon-shaped vaginal imprint area (Wheeler, 1889) with trypsin digestion (50 mg ml<sup>-1</sup> porcine pancreas type IX, EC 3.4.21.4, Sigma) in phosphate buffer (pH 7.27) at 25°C for 24 h, followed by sonication (10 min) and a second 24 h digestion cycle. The trypsinized covering was rinsed several times with buffer, dried, mounted onto metal stubs, coated, and observed using SEM (as described above).

Confocal microscopy

A 2–3 mm<sup>2</sup> section of the proximal end, including the escutcheon-shaped vaginal imprint area, was dissected from each oothecal covering. Embryonic tissues were removed, leaving behind the chorion, which tightly adheres to the internal surface of the covering. Samples were then placed on a glass slide, surface stained with 1 µl fluorescein (5 mg ml<sup>-1</sup>) and sealed in place with a coverslip. The preparations were observed on a Zeiss LSM 510 Laser Scanning Microscope (software version 2.5) using a Plan-Apochromat 100× objective, 488 nm argon laser and a BP 505–550 nm filter. A

Table 1. Distribution of radiolabel in oothecae attached to female *Blattella germanica* after injection with <sup>3</sup>H<sub>2</sub>O

Time post injection <sup>1</sup> (h)	N	% Radiolabel <sup>2</sup>		
		Female	Oothecal quadrants	Oothecal total
0.5	3			0.41±0.28
2	3			2.41±0.91
6	3			4.19±2.15
24	3			18.07±8.16

<sup>1</sup>Females were injected with 16,199±882 Bq.

<sup>2</sup>Percentage of radiolabel at each incubation interval was based on the total radioactivity in the female and oothecal components: 100% at 0.5 h, 13,651±877 Bq (84% of injected dose); at 2 h, 15,067±973 Bq (93% of injected dose); at 6 h, 12,882±914 Bq (79% of injected dose); and at 24 h, 12,193±1,146 Bq (75% of injected dose).

5.6 μm stack was collected, and a three-dimensional projection of the stack was generated.

**Results**

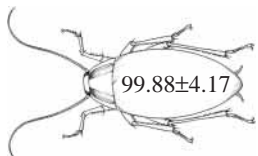
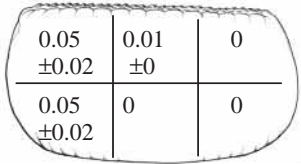
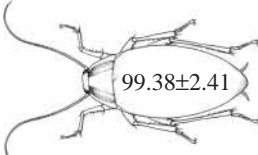
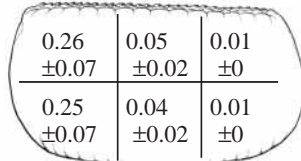
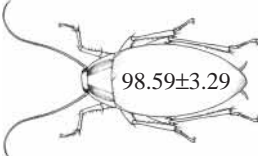
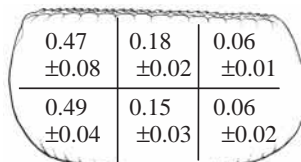
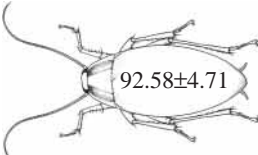
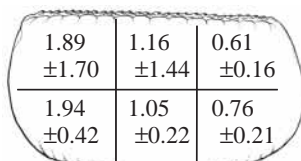
Tritiated water was used to monitor water movement between females and their oothecae. It was assumed that the isotopic effects and potential proton exchanges with other molecules were minimal and that movement of tritium could be used to assess movement within water compartments (Halleman et al., 1982; Kunes, 1989; Robertshaw, 1982).

Table 1 illustrates the distribution of <sup>3</sup>H<sub>2</sub>O from hydrated females (W) through their oothecae over a 24 h period. Similar distribution of radiolabel through oothecae from water-deprived females (W/O) is provided in Table 2. Comparisons within groups (W and W/O) were based on the total amount of tritium detected in each female/oothecal system and are reported as the percentage of activity within each of the components. The rate of radiolabel remaining in the system

after 24 h was 75% (W) and 65% (W/O) of the injected dose, respectively (Tables 1, 2). Results indicate that over time, <sup>3</sup>H<sub>2</sub>O moves from females through the proximal to the distal ends of their oothecae. Higher levels of activity were always found in the proximal end. Activity was distributed more or less evenly between the respective lateral quadrants/sextants of the oothecae. A comparison of the total amount of <sup>3</sup>H<sub>2</sub>O contained in the oothecae from the W/O females (7.5%; Table 2) compared with that in the W females (18%, Table 1) 24 h after injection was significant [(P=0.01; analysis of variance (ANOVA)], indicating water availability to the female might influence the water-transfer process(es).

SEM clearly revealed differences between the distal (Fig. 2A) and the proximal (Fig. 2B) ends of *B. germanica* oothecae. The distal end has a relatively smooth surface, but the proximal end contains an ‘escutcheon-shaped vaginal imprint’ with ‘delicate wrinkles’ first described by Wheeler (1889). Increasing the magnification of the wrinkled region surrounding the vaginal imprint area revealed that this region

Table 2. Distribution of radiolabel in oothecae attached to water-deprived female *Blattella germanica* after injection with <sup>3</sup>H<sub>2</sub>O

Time post injection <sup>1</sup> (h)	N	% Radiolabel <sup>2</sup>			
		Female	Oothecal sextants		Oothecal total
0.5	5				0.12±0.04
2	5				0.63±0.18
6	5				1.43±0.18
24	7				7.45±1.17

<sup>1</sup>Females were injected with 16,448±999 Bq.

<sup>2</sup>Percentage of radiolabel at each incubation interval was based on the total radioactivity in the female and oothecal components: 100% at 0.5 h, 16,119±751 Bq (98% of injected dose); at 2 h, 14,860±402 Bq (90% of injected dose); at 6 h, 13,461±501 Bq (82% of injected dose); and 24 h, 10,637±531 Bq (65% of injected dose).

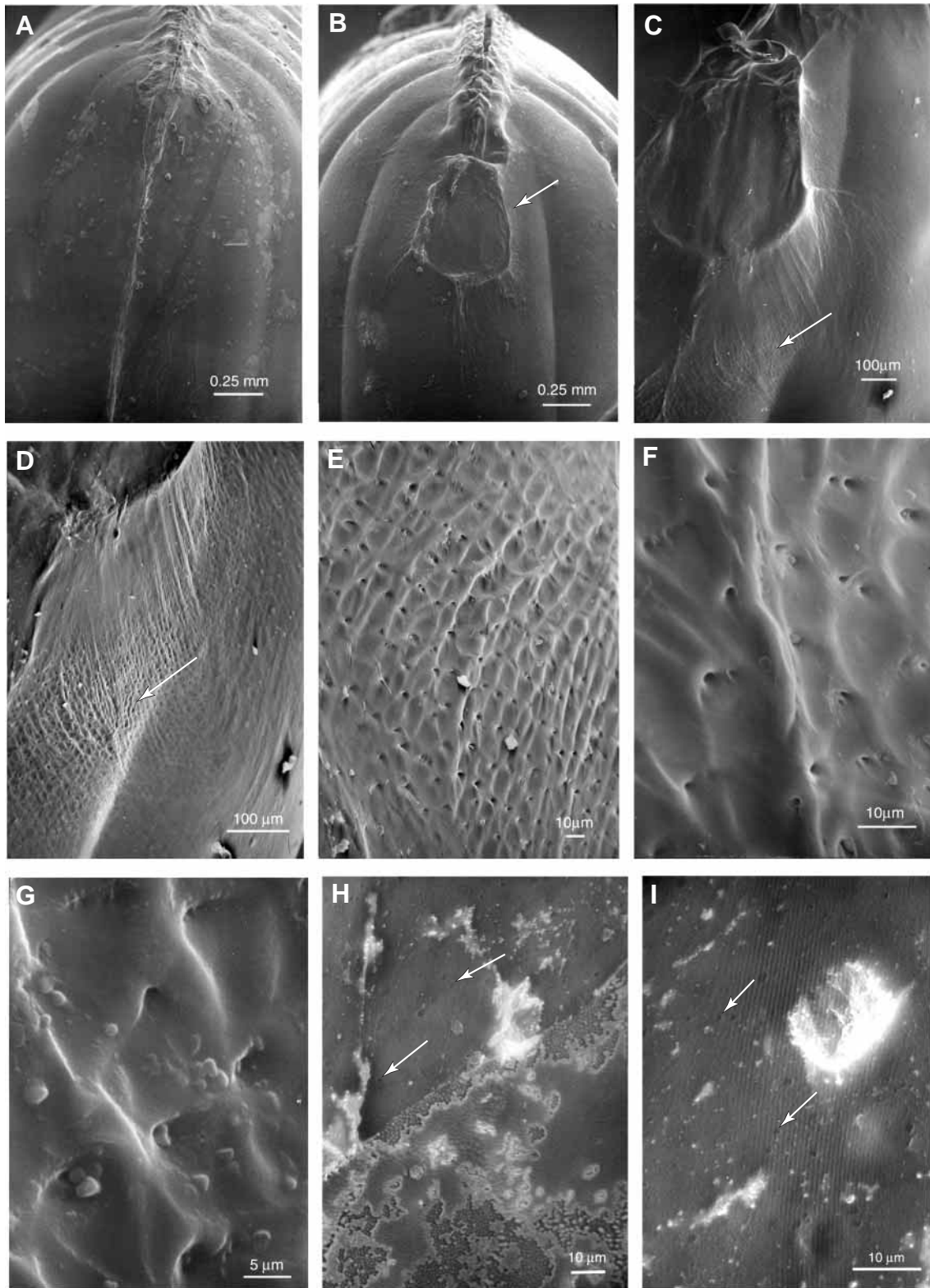


Fig. 2. Scanning electron microscopy images of *Blattella germanica* oothecae. (A) Distal end of an ootheca. Scale bar, 0.25 mm. (B) Proximal end of an ootheca showing the 'escutcheon-shaped' vaginal imprint (arrow). (C) Magnification of the ventro-lateral escutcheon region (arrow indicates the 'pore-field' area). (D) Magnification of 'pore-field' (arrow). (E–G) Increasing magnification of the pore field area shown in D. (H, I) Pores (arrows) revealed on the internal surface of the oothecal covering after chorion removal using trypsin. The pore sizes are approximately 1–2  $\mu\text{m}$  in diameter.

contains an area of small ‘pores’ (approximately 1–2  $\mu\text{m}$  in diameter) that penetrate the exterior of the oothecal covering (Fig. 2C–G). The interior surface of this region of the oothecal covering was also found to contain pores that appear to penetrate the covering (Fig. 2H,I). In order to demonstrate that these pores penetrate completely through the oothecal covering, we used a fluorescent stain that coated the external and internal surfaces of the oothecal covering and a confocal microscopic system to examine these two oothecal surfaces simultaneously. Confocal images generated by optically scanning through a ventrolateral section adjacent to the escutcheon confirmed that some of these pores do indeed penetrate the oothecal covering (Fig. 3A–L). A three-

dimensional image showing proximal and distal aspects by rotation of a reconstructed confocal stack also demonstrated that the pores are contiguous (as represented by the black holes) with both the external and internal surfaces (Fig. 3M–Q).

To examine the permeability of water and water-soluble materials across the oothecal covering, a microparabiotic chamber was developed. Differences in water permeability between the distal and proximal ends of the oothecae were observed during preliminary experiments using this chamber. In these experiments, sections of the oothecal covering from distal and proximal ends of the oothecae were incubated for 24 h in the microparabiotic chamber assembly. A permeability

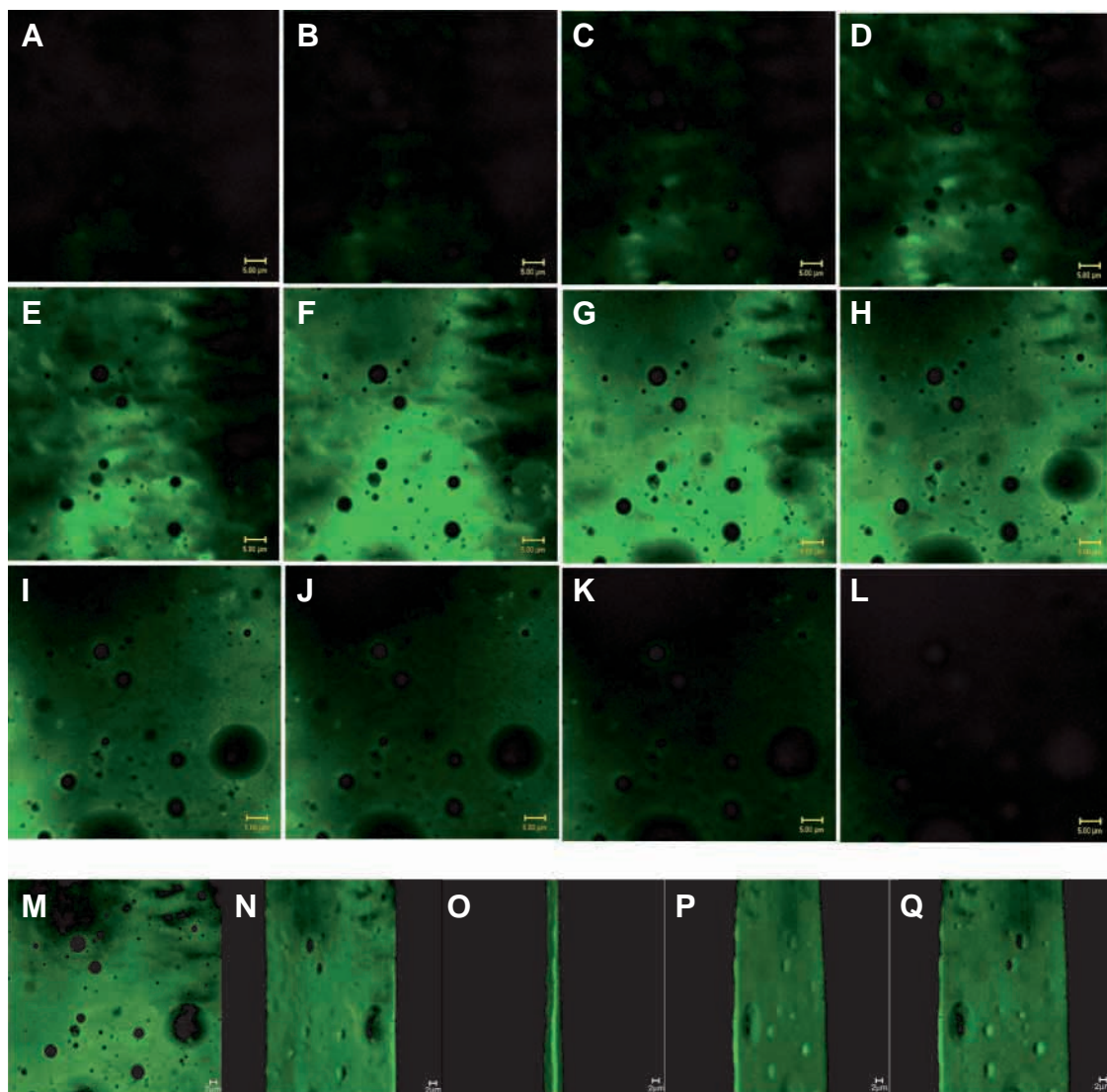


Fig. 3. Confocal images of the oothecal pore-field area. These images were generated by optically scanning through a ventro–lateral oothecal section of the pore-field area (Fig. 2D–I). Prior to viewing, 1  $\mu\text{l}$  of fluorescein dye (5  $\text{mg ml}^{-1}$ ) was applied to the tissue preparation to show fluorescent contrast on the oothecal surfaces, enabling visualization of the pores (as represented by the black holes) through the oothecal matrix. These images were gathered at 0.26  $\mu\text{m}$  intervals from the exterior to the interior surfaces of the oothecal covering. (A–L) The optical sections taken, beginning below the surface (A), through the matrix, to the field above the inside surface (L). Scale bars, 5  $\mu\text{m}$ . (M–Q) Selected images obtained from projection of the confocal stack, which show the three-dimensional aspect of the covering. Scale bars, 2  $\mu\text{m}$ .

of <5% of initial radioactivity was observed in distal samples compared with a permeability of 42% in proximal end samples. During development of the method, it became apparent that

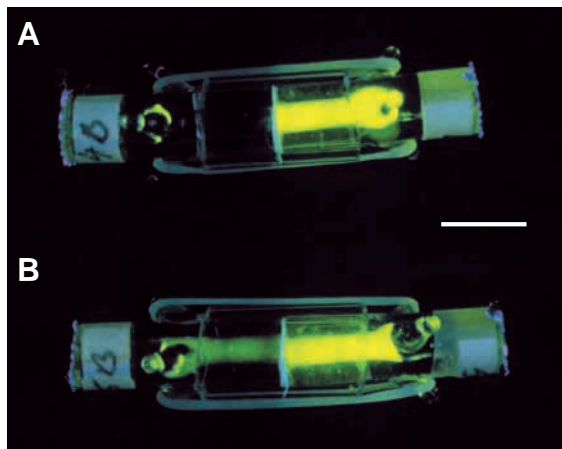


Fig. 4. Fluorescent dye used for detection of buffer solution leaks in microparabiotic chambers. 30µl of buffer was delivered to the internal (left) side of the oothecal tissue, and 30µl of buffer containing a radiolabeled material and fluorescein (50µg ml<sup>-1</sup>) was delivered to the external (right) side of the oothecal tissue prior to incubation. After various incubation times (0.5 h, 2 h, 6 h or 24 h), the microparabiotic chamber was viewed under ultraviolet light (256 nm) to detect leaks. (A) No detectable leakage. (B) Leak detected. Sample replication not used. Scale bar, 1 cm.

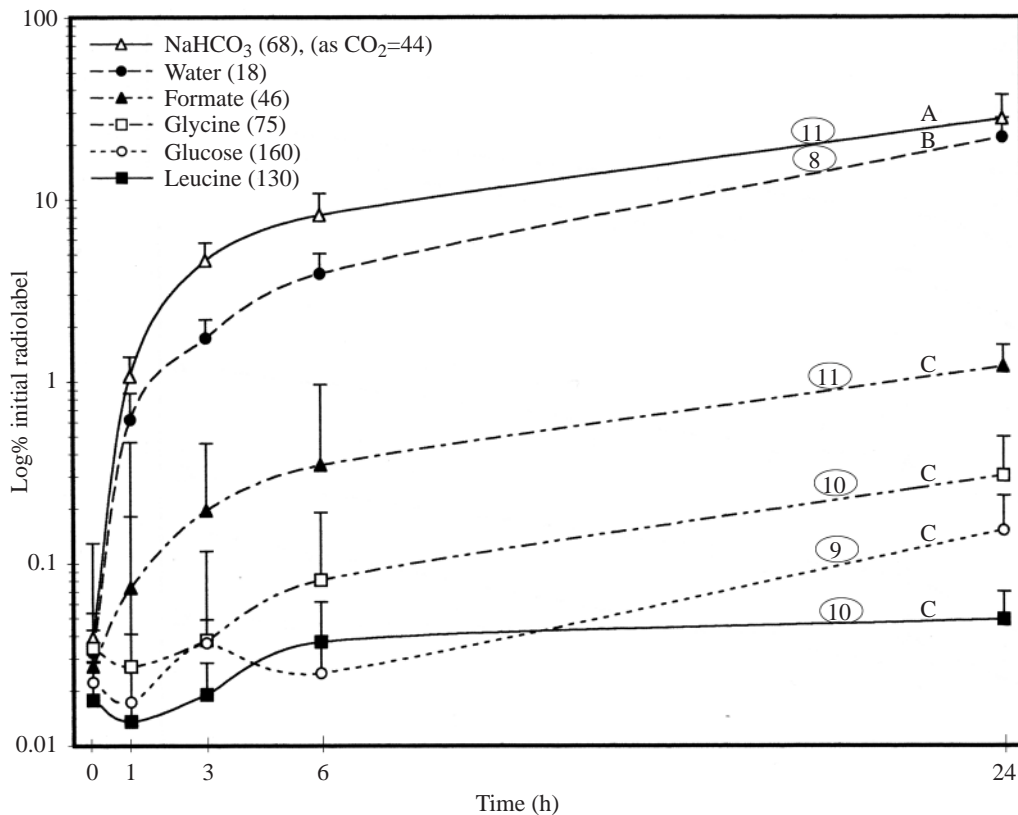
fluorescent dye was needed to ensure that leaks around the edges of the parafilm/tissue could be detected (Fig. 4). Samples showing evidence of leakage were excluded from data tabulations.

Results from experiments designed to compare the permeability of the oothecal escutcheon region to various radiolabeled water-soluble materials are presented in Fig. 5. These microparabiotic assays indicated that, although there were differences in permeability rate, the escutcheon region was permeable to all of the materials tested. In general, molecules with higher molecular weights (leucine, glucose, glycine and formate) showed significantly less movement across the escutcheon than did water or bicarbonate.

Discussion

There is considerable evidence that oothecal retention gives the female the opportunity to influence water balance of her ootheca. In studies based on oothecal mass changes, Roth and Willis (1955c) found that water content increased over time (62% in 4-day-old oothecae, reaching 76% at hatching). By sealing either the proximal or distal halves of manually detached oothecae and monitoring mass loss, Roth and Willis (1955a) demonstrated that the proximal end was much more permeable to water. They also observed that detached oothecae gained 8.3% of their initial mass when their proximal ends were placed in contact with moist filter paper, whereas the oothecae lost 6.1% of their initial mass when the distal ends

Fig. 5. Comparison of movement of various water-soluble materials across the escutcheon region of the proximal end of 0-1-week-old oothecae using microparabiotic chambers (Figs 1, 4). Comparisons of the rate of isotope movement from the exterior side to the interior side of the oothecal surface were measured using 1µl samples taken at 0.5 h, 2 h, 6 h and 24 h incubation times. Radioactivity recovered on the inside of the oothecal covering is reported as log% of the initial radiolabel placed on the exterior side of the covering. Molecular weights (Da) of the isotopes used are shown in parentheses. The number of replications (N) used for data analysis is shown for each material. Statistical comparisons of the means were done using ANCOVA (analysis of covariance); different letters associated with the curves indicate significant differences at the P=0.05 level.



were placed in contact with moist filter paper. Roth and Willis (1955b) found that egg hatching in detached oothecae depends on the rate of water loss, the amount of water present when the oothecae are removed and the amount of time remaining before complete embryogenesis. These observations were confirmed by Barson and Renn (1983), who reported that, when manually detached oothecae were  $\leq 16$  days, they were less likely to hatch than those that were 21 days old, while those held at 45% relative humidity were less likely to hatch than those maintained in a 70% relative-humidity environment. We characterized water movement rates from females to their oothecae, and the distribution of the  $^3\text{H}_2\text{O}$  from injected females to their oothecae, during a 24 h period (Tables 1, 2). In both experiments, the radiolabel moved from the proximal to the distal end, essentially following a concentration gradient of  $^3\text{H}_2\text{O}/\text{H}_2\text{O}$  within the oothecae. Transfer rates appeared to be influenced by the water balance of the females. Those deprived of water 24 h before  $^3\text{H}_2\text{O}$  injection (W/O females) transferred less radiolabel to their oothecae (7.5%; Table 2) than those that had not been water deprived (W females; 18%; Table 1). This indicates female conservation of limited water resources.

The striking physical differences between the proximal and distal ends of *B. germanica* oothecae have been observed by numerous workers (Lawson, 1951; Roth and Willis, 1954, 1955a; Wheeler, 1889). The distal end of the ootheca is much darker and more highly sclerotized than the more-flexible, unsclerotized proximal end, which can be almost white in color. This obvious difference between the two ends led to studies on differential permeability, water transfer and their relationship with relative humidity (Roth and Willis, 1955a,b; Barson and Renn, 1983). However, to our knowledge, no-one has investigated the structural differences between the two ends of the oothecae in any detail, although Wheeler (1889) did report that the proximal end of the oothecae typically contained an 'escutcheon-shaped vaginal imprint'. We have shown that the lateral ventral margins of the external escutcheon contain pores of approximately 1–2  $\mu\text{m}$  in diameter (Fig. 2C–G). Furthermore, we have confirmed that these pores penetrate the oothecal covering (Figs 2, 3).

The discovery of the pore field associated with the escutcheon supports observations that the proximal end of the ootheca is more permeable to water. Experiments using a microparabolic chamber with radiolabeled materials indicate that the escutcheon region is not only permeable to water but also to low-molecular-weight water-soluble materials (Fig. 5). This raises the possibility that materials other than water might be transferred across the proximal ends of the oothecae. In preliminary experiments, we found detectable quantities of radioactivity in hatched nymphs from oothecae carried by gravid females that had been injected with either radiolabeled glucose or leucine (D. E. Mullins and K. R. Tignor, unpublished data). Additional work that might indicate a mechanism for maternal investment in the oothecal development of two other cockroach species (*Byrostria fumigata* and *Gromphadorhina portentosa*) was done by Snart

et al., who discovered pores (approximately 1  $\mu\text{m}$  in diameter) on papillae projecting from the luminal surface of female brood sacs (Snart et al., 1984a). Ultrastructural examination of these papillae revealed the presence of 'glandular units' associated with a duct that opens at a pore on the cuticularised apex of the papillae (Snart et al., 1984b). This work raises some interesting questions on the physiological, and perhaps nutritional, relationships that occur between the female and the embryos that she carries in her brood sac during gestation/embryogenesis.

There has been considerable work on the reproduction biology of the German cockroach that includes both preovipositional and postovipositional aspects. Preovipositional studies include vitellogenesis (Martin et al., 1998), hormonal regulation of oogenesis (Schal et al., 1993; Holbrook et al., 2000; Vilaplana, 1999), nutritional requirements associated with reproduction (Cochran, 1983; Kunkel, 1966) and maternal and paternal investment (Mullins et al., 1992). Postovipositional (postovulation) investment and parental care has been reviewed by Nalepa and Bell (1997). German cockroaches appear to represent an important link in the evolutionary transition from oviparity to ovoviviparity (Nalepa and Bell, 1997; Roth and Willis, 1954). The process of oothecal formation is quite complex, including chorion production, orientation and alignment of the eggs, their encapsulation within the oothecae and elaboration of the keel (Tanaka, 1976; Roth and Willis, 1954; Wheeler, 1889). Formation and protrusion of the oothecae is followed by a 90° rotation and retention of the oothecae until after the time of hatching (Tanaka, 1976; Roth and Willis, 1954; Wheeler, 1889).

The attachment of the oothecae to the female during embryogenesis is thought to be an initial step in development of ovoviviparity, a process inclusive of oothecal formation followed by internalization within the female. *B. germanica* may indeed represent an important intermediate stage before internalization of the oothecae; there are at least four known species of blattids that reproduce by ovoviviparity (Roth, 1997). Ovoviviparity (leading to viviparity) is thought to have appeared as an evolutionary response for protection from biotic factors (mortality from predation, pathogens, parasites, cannibalism), abiotic factors (avoidance of physical extremes such as temperature, humidity, etc.) and selection of a suitable habitat for the nymphs at the hatch. This is followed by development of postovipositional support in terms of water exchange (ovoviviparity) and provision of nutrients during embryogenesis (viviparity) (Nalepa and Bell, 1997).

The work presented here also raises some questions regarding the female–embryo relationship(s). We have not yet examined the female vestibular structures that are in contact with the escutcheon while it is carried by the female. However, it is clear that the female genitalia clasp the oothecae quite firmly (Fig. 6), and the vestibulum that is associated with the proximal end of the ootheca appears to be membranous and capable of providing an environment in which water can be efficiently transported (Fig. 7). Fig. 2B shows that the pore-





Fig. 6. Photograph demonstrating the degree of sclerotization of the oothecae; the distal end of the ootheca is darker (more sclerotized) than the proximal end (less sclerotized). This photograph also shows the close structural relationship between the gravid female and her oothecae. Scale bar, 1 mm.

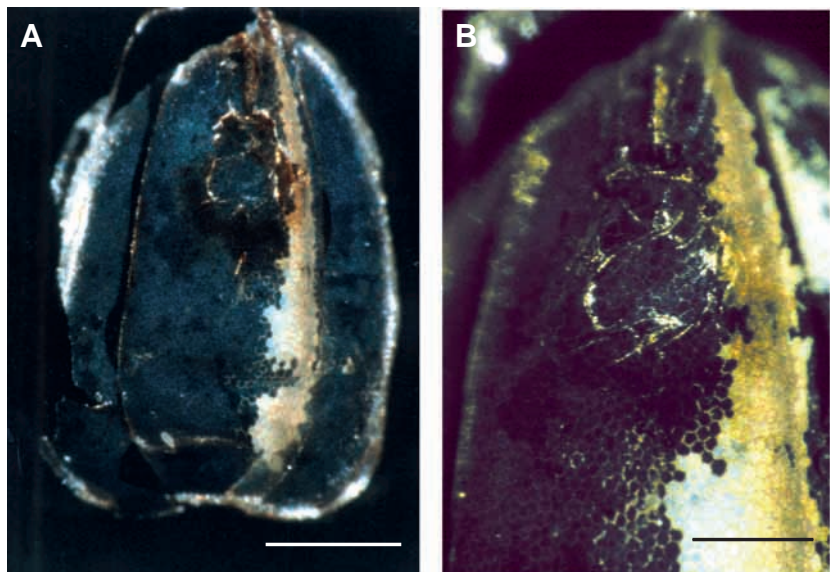
field area associated with the perimeter of the escutcheon is about  $0.5 \text{ mm}^2$ . The female genital area, which includes the vestibulum, appears to be large enough ( $1 \text{ mm} \times 2 \text{ mm}$ ; Fig. 6) to provide sufficient surface area for oothecal contact with the membranes lining the internal surfaces of the vestibulum ( $1 \text{ mm} \times 2 \text{ mm}$ ; Fig. 7), including the pore-field area of the ootheca. Close examination of the female vestibulum might provide useful information on the structural basis for liquid transport between the female and the oothecae that she carries.

Fig. 8. Light micrographs of the proximal end of an empty ootheca obtained using a Wild MP400 Photomakroskop equipped for dark-field illumination.  $5\text{--}10 \mu\text{l}$  water was applied to the chorion (inner surface), and observations were made as the water evaporated from the specimen. The presence of water is revealed by the birefringent or light areas, where the water was retained. Note that the hexagon-shaped structures, characteristic of the chorion, contain water in their internal (ring-like) structures as well as along the exterior or planar surfaces. (A) Scale bar, 1 mm. (B) Scale bar, 0.5 mm.



Fig. 7. The female vestibulum after removal of a 1-week-old ootheca, showing the extent of membranous surfaces that may be involved in water transport. Scale bar, 1 mm.

Another aspect regarding water transport relates to the mechanisms that are involved within the ootheca itself. Transport is most likely achieved by the chorion, but detail on how it is done is lacking. Also, there are differences in interpretation of the structural architecture that have not been resolved (Hinton, 1981). These differences appear to reside in determinations of the composition and arrangement of the components that comprise the hexagonal structure of the chorion. Debate continues as to whether or not they are air filled (Lawson, 1951; Wigglesworth and Beament, 1950; Wheeler, 1889; Hinton, 1981). There is general agreement that the chorion contains air-filled spaces that facilitate respiratory activities within the ootheca. Access for respiratory exchange is through the spongy (white) bodies that are apical extensions of the chorion and become isolated in the keel (Hinton, 1981). However, Lawson (1951) reported on an 'interesting sidelight' of his investigations resulting from the application of dyed oil; he found that when dyed oil was applied near spongy (white) bodies, it diffused from this area and gathered at the posterior ends of the eggs. This observation supports the hypothesis that,



in addition to gas transport and exchange, the oothecal chorion, which envelopes each of the embryos, might also provide for distribution of liquid. Fig. 8 demonstrates that water can be retained by both the hexagonal borders and by the internal structures. Close examination of Fig. 8B shows the pattern of water content as the water evaporates; the interior portions of the hexagons clearing (dark areas) before those areas within the hexagonal borders.

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