Pathological effects of *Pseudodiplorchis americanus* (Monogenea: Polystomatidae) on the lung epithelium of its host, *Scaphiopus couchii*

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SUMMARY

Infection of the desert toad, *Scaphiopus couchii*, by the monogenean *Pseudodiplorchis americanus* involves 2 principal sites: post-invasion juveniles reside in the respiratory tract for 1 month before migrating to the urinary bladder where they reach sexual maturity and may live up to 4 years. While previous work has demonstrated the long-term impact on host condition of the blood-feeding adults, this study assesses pathological effects of the short-term pulmonary infection. Lung ultrastructure was compared in toads (i) maintained in captivity for 1 year without invasion, and (ii) experimentally infected with 50–300 juveniles/host, equivalent to burdens in the wild, and examined 23–44 days p.i. Typically, the alveolar lining of *S. couchii* is composed of a single cell type with characteristics of both Type I and Type II pneumocytes. However, infected lung tissue exhibited an inflammatory reaction with epithelial cell vacuolation, interstitial oedema, and an increase of alveolar exudate, leucocytes and fibrous tissue. Accompanying a post-infection increase in host immune cells in the lungs, there was evidence of reciprocal tegumental damage to the parasites. Lung epithelium of toads free of infection for 1 year exhibited scar tissue representing a residual effect of past infection. The pathological consequences of *P. americanus* infection therefore have 2 components. Acute lung infection coincides with the host's brief activity season: impaired respiratory function could compromise feeding and accumulation of reserves and hence ability to survive following a 10 month period of hibernation. Additionally, adult toads are normally exposed annually to re-infection and may accumulate chronic lung damage with extended effects on host survival.

Key words: ultrastructure, pathology, immunity, leucocyte, alveoli, Amphibia, Monogenea.

INTRODUCTION

The monogenean *Pseudodiplorchis americanus* infects an unusually wide range of organ systems within its host, the North American toad, *Scaphiopus couchii*: the oral cavity and associated passages including nostrils and vocal sacs, the lungs, stomach, intestine and urinary bladder (Tinsley & Earle, 1983; Tinsley & Jackson, 1986). The desert environment in which this host–parasite association occurs imposes a strict compartmentalization on life-cycle processes. This has facilitated studies providing a more comprehensive insight into the dynamics of the host–parasite interaction than recorded for most other monogeneans (Tinsley, 1995, 1999). Hosts hibernate for 10–11 months each year in burrows a metre below the soil surface and, for this major segment of the year, populations are free of all platyhelminth invasion. The toads emerge during the short season of monsoon rains in July/August and adults enter newly-formed pools to spawn, a process restricted to a few hours on only 1–3 nights/year. At this time, gravid *P. americanus* in the host's urinary bladder release infective larvae. This behaviour precisely constrains waterborne transmission involving monogenean oncomiracidia, but the brief episode typically results in a prevalence amongst mating males of 100% and mean intensity around 100 worms/host (Tinsley & Jackson, 1988). Invading larvae enter the respiratory tract via the nostrils and then pass to the lungs 1 week p.i. where they remain for 3–4 weeks before returning to the mouth and migrating through the gut to the urinary bladder. The worms mature in the bladder and accumulate infective larvae *in utero* in preparation for the next transmission opportunity, 1 year later. However, infection levels appear to be strongly regulated and by the end of host hibernation prevalence has fallen to 50% and mean intensity to around 6 adult worms/host (Tinsley & Jackson, 2002).

The strict ecological constraints operating on both host and parasite highlight the potential influence of parasite-induced pathology on survival in a desert environment, creating a conflict that is greater than in most host–parasite systems. The characteristics defining the *P. americanus*–*S. couchii* interaction are unusual. For the 10–11 months of hibernation the host represents a closed system: the toads do not feed and all energetic needs must be met from reserves accumulated during the preceding activity

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season and stored principally as lipid. Throughout this period, consumption of host blood by *P. americanus* for growth and reproduction translates into a drain on the reserves laid down for host survival, without any opportunity for replenishment. *P. americanus* may live for up to 4 years and each summer a new cohort of parasites may be recruited contributing to a succession of age classes within each host and an accumulating cost on resources (Tinsley, 1999).

Host ecology imposes a severe restriction on opportunities for parasite transmission and only 4 other helminths exploit this amphibian, each with a prevalence of $< 5\%$ and very low intensities (Tinsley, 1995). The fact that *P. americanus* is the only significant helminth infection of *S. couchii* simplifies measurement and interpretation of pathogenic effects. Parasite blood consumption has been determined quantitatively (Tocque & Tinsley, 1992) and this has a density-dependent and temperature-dependent negative effect on host condition. In toads examined at the end of hibernation, fat reserves were negatively correlated with parasite intensity. Tocque (1993) calculated that resource depletion by an average burden of *P. americanus* is equivalent to about 7% of the lipid required for *S. couchii* to survive for 1 year. In females (which bear the extra cost of ovary production), there is also a significant reduction in red blood cell levels. These parasite-induced pathogenic effects have been reproduced in laboratory experiments under controlled environmental conditions (Tocque & Tinsley, 1994*a*). The studies provide a clear demonstration of the potential for parasite-induced mortality: infected toads entering hibernation without the required 7% surplus of stored lipid, above that for their own needs, might be expected to die. However, the actual outcome depends on a range of other factors in addition to worm burden, especially the success of host feeding and energetic investment during the previous season, and the environmental conditions and duration of the hibernation (and starvation) period (Tinsley, 1999).

This phase in the host–parasite interaction, involving relatively long-lived parasites creating a chronic drain on host resources, is well documented (Tinsley, 1995), but there is little information on the pathogenic consequences of juvenile *P. americanus* in the respiratory system. Parasite infection in the pulmonary tract is normally short lived, with migration to the bladder occurring around 4 weeks p.i., and the overall impact of this phase might therefore be limited. However, the period of pulmonary infection coincides more or less exactly with the host's activity season when the nocturnal toads feed intensively on desert invertebrates (Tocque, Tinsley & Lamb, 1995). Up to 400 worms/host may occur in the lungs of naturally infected toads (Tinsley, 1989): a direct effect on host physiology might be predicted, associated potentially with impaired respiratory efficiency. Any reduction in foraging ability could have serious implications if the host is prevented from accumulating sufficient reserves to survive through the following 10 months starvation. At field study sites in Arizona, *S. couchii* are almost invariably infected by *P. americanus* during each annual mating period (Tinsley, 1999), so any pathological sequelae are likely to recur every year.

In this study, to assess pathogenic effects on the lung epithelium, field-caught *S. couchii* were experimentally infected with *P. americanus*, and lung ultrastructure was compared in toads experiencing an acute infection with those maintained in captivity for 1 year without opportunity for parasite invasion.

MATERIALS AND METHODS

Collection of material and experimental infections

Scaphiopus couchii (59–66 mm snout–vent length) were collected in the San Simon valley, southeast Arizona, and experimentally infected with *Pseudodiplorchis americanus* larvae following the procedures outlined by Tinsley & Earle (1983). Hosts were anaesthetized by immersion in 1% MS222 (Sandoz) and killed 23–44 days post-exposure. Upper and lower parts of the head, lungs, gut and bladder were examined for parasites. The right lung tissue was teased apart with fine forceps and the number of parasites counted, while the left lung was fixed intact for electron microscopy. Similar numbers of worms would be expected in the left and right lungs of infected *S. couchii* (unpublished studies). Other animals, transported to the UK after being experimentally infected, were fed for 3–6 weeks at 22–28 °C and then maintained in hibernation at 10 °C (see Tinsley & Earle, 1983). After 12 months without opportunity for further oncomiracidial invasion, some toads were dissected immediately following arousal from hibernation and lung tissue fixed; others were re-infected and then dissected and fixed 24 days p.i. These latter hosts provide a repeat of the acute infection induced after field collection and a control for potential effects of 1 year's maintenance in the laboratory. The infection levels recorded (50–300 juveniles/host, see below) accord closely with worm burdens observed after natural exposure in Arizona (see Tinsley, 1999).

Transmission electron microscopy

Lungs were fixed in 2·5% glutaraldehyde buffered with 0·1 M sodium cacodylate (pH 7·2) for 4 h and washed overnight in buffer. Tissue was post-fixed for 10 min in 1% buffered osmium tetroxide, washed in buffer and cut into 2×4 mm blocks before post-fixing for a further 1 h. After dehydration in graded ethanols and clearing in propylene oxide, specimens

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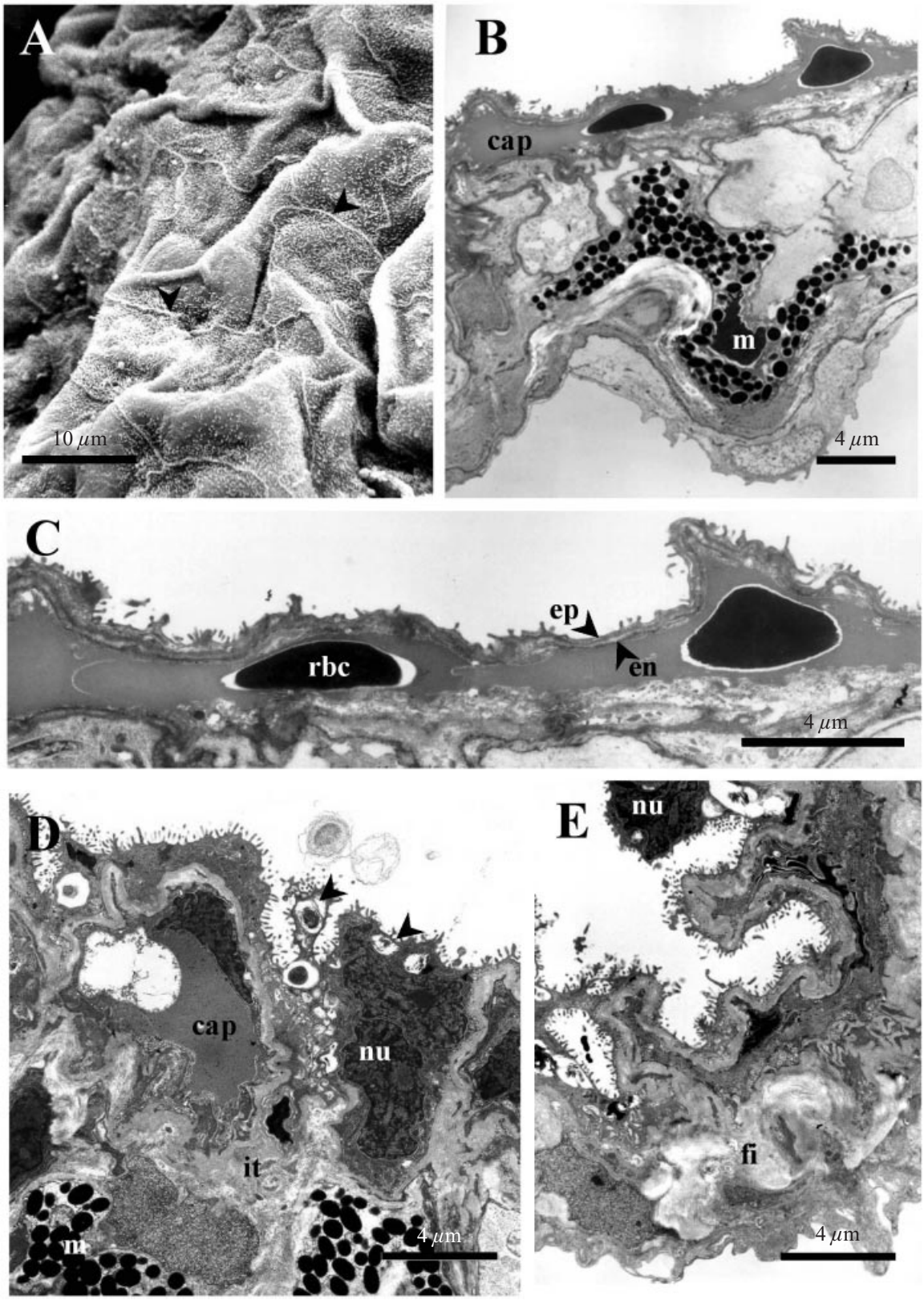

Fig. 1. Lung tissue of *Scaphiopus couchii* free of *Pseudodiplorchis americanus* invasion for at least 1 year. (A) SEM. Microvilli and cell junctions (arrowheads) at surface of lung epithelium. (B–E) TEM. The epithelium is composed of a single cell type that bears numerous microvilli; capillaries (cap) containing red blood cells (rbc) underlie the basal lamina of the epithelium; melanocyte (m) dendritic processes extend throughout the interstitium (it). (C) Higher magnification of (B) showing the thin air/blood interface where basal lamina of epithelium (ep) and endothelium (en) are fused. (D) Prominent epithelial nucleus (nu) in cell that resembles Type II pneumocyte; vesicles and multilamellate bodies (arrowheads) in epithelium. (E) Damaged lung epithelium (scar tissue); thickened alveolar septa with prominent fibrous layer (fi); nucleus in epithelial layer (nu); notable absence of capillaries.

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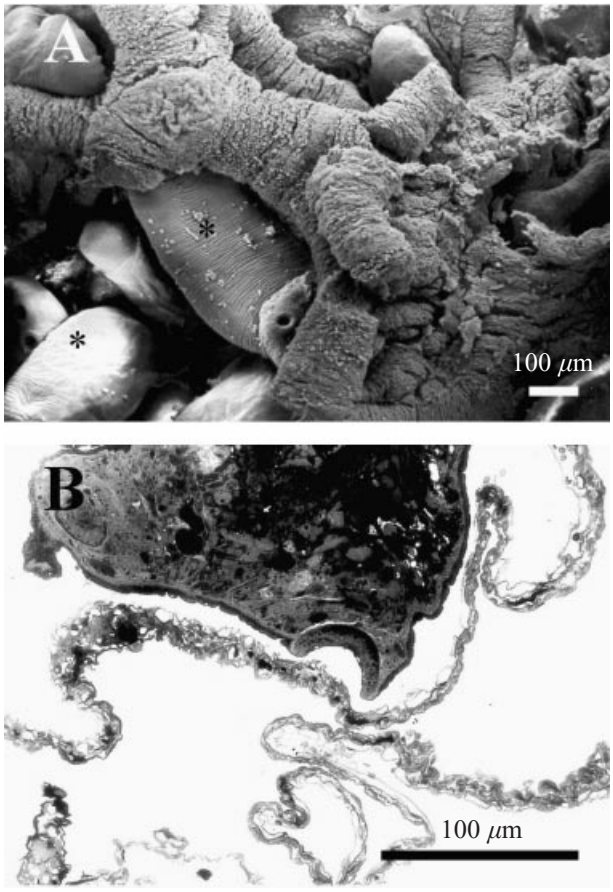

Fig. 2. *Pseudodiplorchis americanus* juveniles, 23 days p.i. (A) SEM of parasites (asterisks) attached to lung epithelium. (B) Light micrograph of toluidine blue stained section of posterior region of a worm surrounded by folds of vacuolated lung epithelium.

were infiltrated and embedded in Araldite resin. Semi-thin sections stained with toluidine blue were examined by light microscopy. Ultra-thin sections were double-stained with uranyl acetate and lead citrate and viewed in a JEOL 1200 EX electron microscope operated at 80 kV. At least 3 tissue blocks were examined from each toad exposed to experimental infection ($n = 6$) and from each of those maintained for 1 year without further infection ($n = 3$). In addition to parasites fixed *in situ*, ultra-thin sections were examined from 5 worms that had been dislodged from lung tissue during processing.

Scanning electron microscopy

Following post-fixation in osmium tetroxide, lung tissue was frozen in liquid N_2 (EMscope sputter cryo-system) and viewed at low temperature in a PHILIPS 500B scanning electron microscope (SEM) operating at 10 kV. Other freshly-fixed specimens and some of the cryo-specimens were dehydrated in acetone and soaked in amyl acetate. This material was dried using CO_2 in a critical point drier, sputter coated with a gold-palladium mixture and viewed with SEM at ambient temperature at an accelerating voltage of 25 kV.

RESULTS

The lungs of *Scaphiopus couchii* are simple air sacs directly connected to the glottis. Internally, the pulmonary membrane of each lung is greatly folded with the alveoli separated by highly vascularized septa: ultrastructural observations were restricted to the alveoli. The lung epithelium is similar in morphology to that of other amphibians in which either 1 or 2 cell types, equivalent to Type I and Type II pneumocytes, have been described (Welsch, 1981). In *S. couchii*, the alveolar lining appears to consist of a single cell type that forms a flattened epithelial layer connected by tight intercellular junctions (Fig. 1 A). All alveolar cells bear numerous microvilli, and contain vesicles and multi-lamellate bodies (Fig. 1 B–D), thus sharing characteristics of both Type I and Type II pneumocytes. Rarely, cuboidal cells were detected which superficially resemble Type II cells (Fig. 1 C). However, without serial sections, it was not possible to determine whether peripheral regions of these cells extended over adjacent capillaries as in Type I pneumocytes. The lung epithelium is supported by a basal lamina that, in thin regions of the alveolar septa, is fused with the basal lamina of the alveolar epithelium and capillary endothelium. It contains connective tissue (fibroblasts), interstitial fluid and prominent melanocytes with long dendrites packed with melanosomes (Fig. 1 B). Some unidentified cells were detected in the interstitium of uninfected lungs but there were no clearly recognizable leucocytes. Rarely, cell fragments or vesicles that appeared to have been released from the epithelium were detected in the alveolar space of uninfected lungs but there were no free cells in the lumen of these specimens.

Although toads uninfected in the year prior to ultrastructural analysis exhibited no acute pathological effects, there was evidence of tissue damage covering approximately 5 % of the lung surface in 2 of the 3 individuals examined. In the alveolar pockets, some tissue adjacent to the thin vascularized septa was largely devoid of capillaries and contained many fibrils in the interstitium (Fig. 1E). Prominent nuclei surrounded by relatively electron-dense cytoplasm were more frequently observed within this abnormal epithelium.

In toads exposed to *P. americanus* oncomiracidia 23–25 days prior to autopsy, parasite burdens in the lungs (estimated on the basis of equal numbers in both lungs) ranged from 50 to 200 worms/host. Parasites recovered from the 2 hosts infected 44 days p.i. indicated total lung burdens of 160 and 300 worms/host. Most of the worms fixed *in situ* in the lungs became detached during processing but were held in position by folds of the alveolar septa (Fig.

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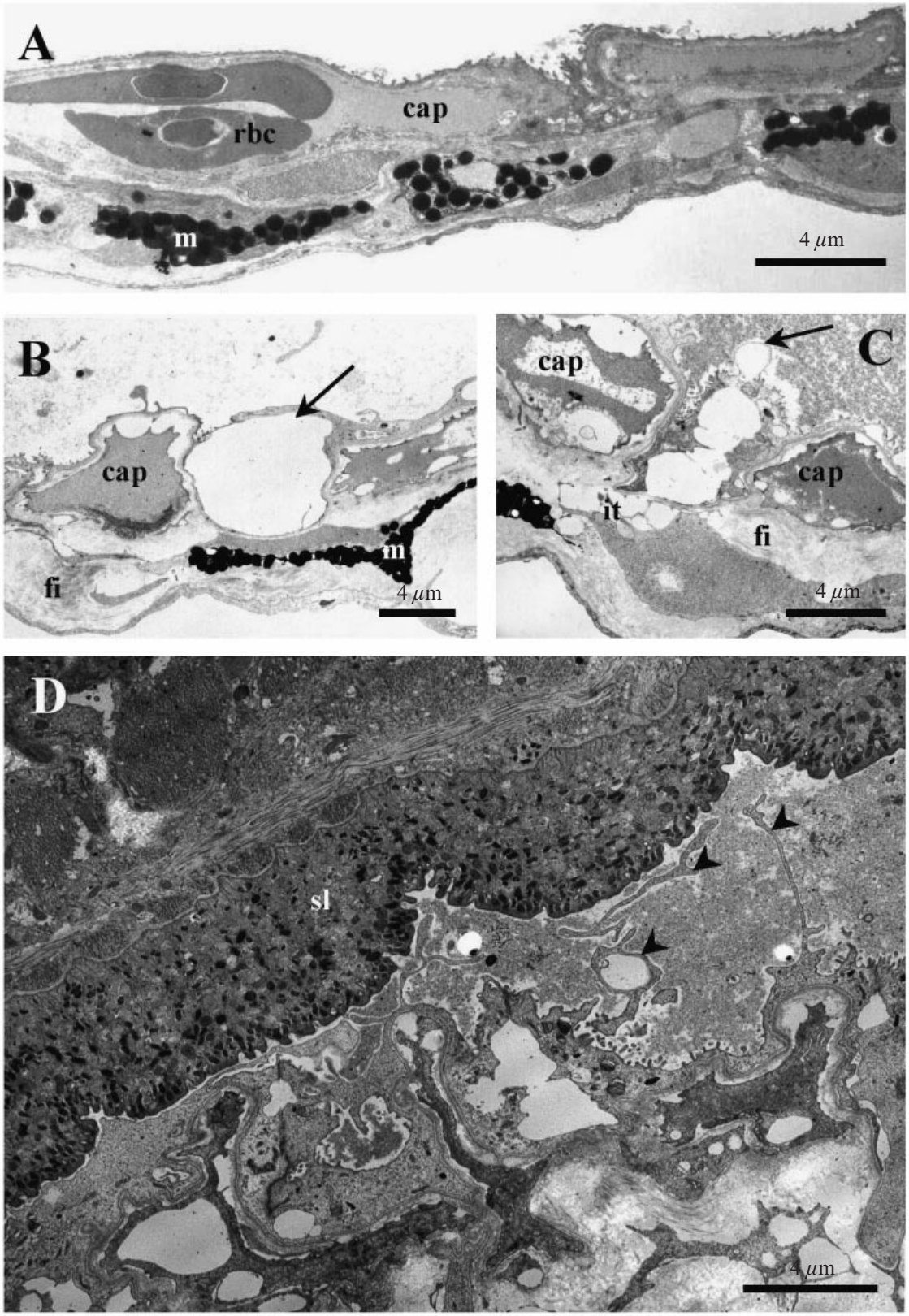

Fig. 3. Lung tissue of *Scaphiopus couchii* experiencing acute infection by *Pseudodiplorchis americanus* (23–25 days p.i.). TEM. (A) Undamaged epithelium overlying capillaries (cap) containing nucleated red blood cells (rbc); melanocytes (m) packed with melanosomes under the basement lamina. (B, C) Prominent vacuoles overlying basal lamina of epithelium causing surface blebing (arrow); smaller vacuoles towards the periphery of capillaries (cap) and within interstitium (it) causing oedema; melanocytes (m); fibrous tissue (fi) also occurs in interstititium. (D) Host lung epithelium in close proximity to surface layer (sl) of parasite tegument; the lung epithelium is highly vacuolated, contains numerous inclusions and the surface membrane is extended into long cytoplasmic processes (arrowheads).

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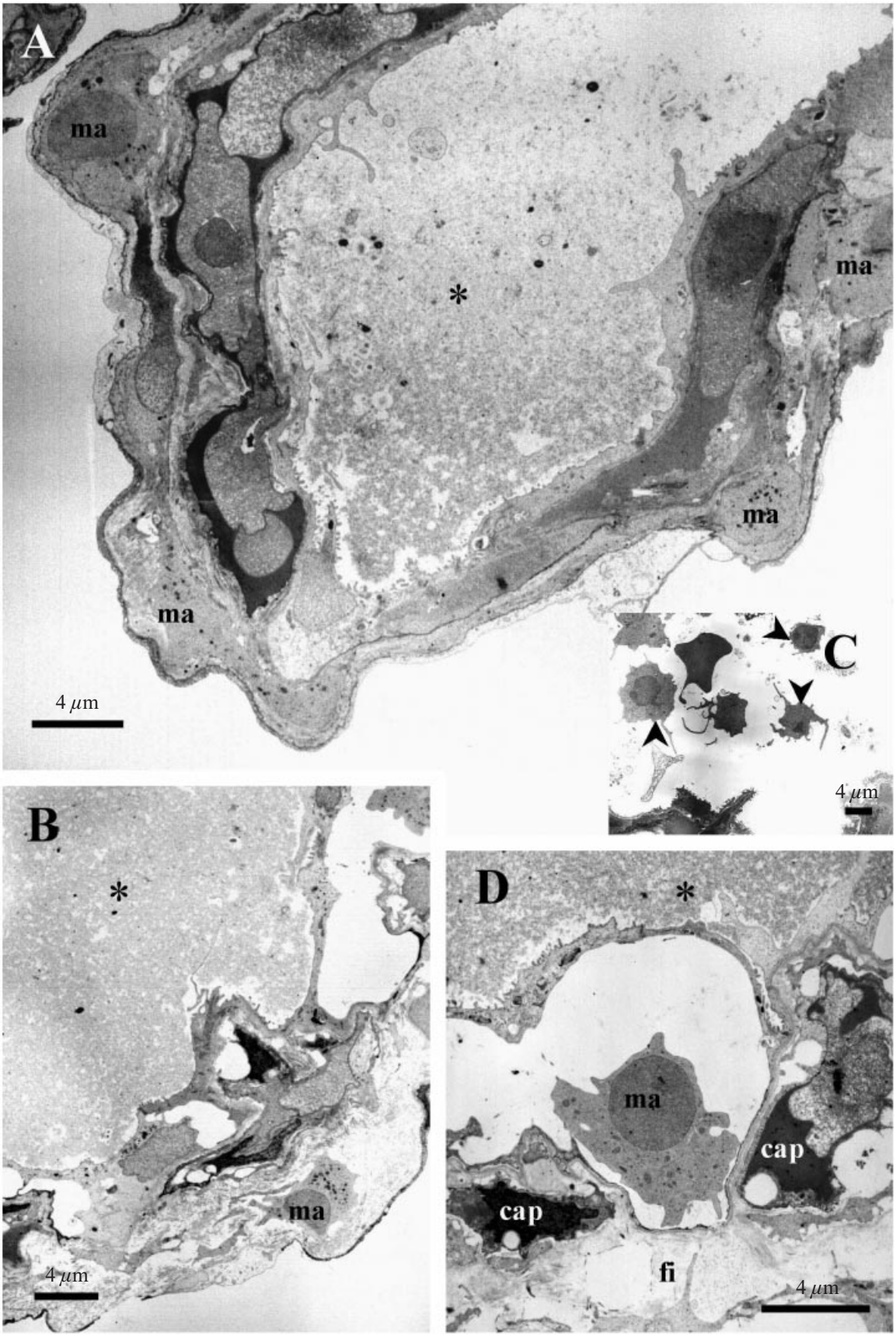

Fig. 4. Lung tissue of *Scaphiopus couchii* infected with *Pseudodiplorchis americanus*. TEM. (A, B) 23 days p.i. Amorphous lung exudate (asterisks) within alveolar pockets; surface membrane of epithelium is extended into cytoplasmic projections and vacuoles are common; putative macrophages (ma) lie within the interstitium. (C) 44 days p.i. Macrophage like-cells (arrowheads) within the alveolar space surrounded by cell fragments. (D) 23 days p.i. Putative macrophage (ma) within a vacuole underlying the lung epithelium; smaller vacuoles disrupting other tissues including capillaries (cap) and fibrous tissue (fi) within the interstitium; prominent lung exudate (asterisk).

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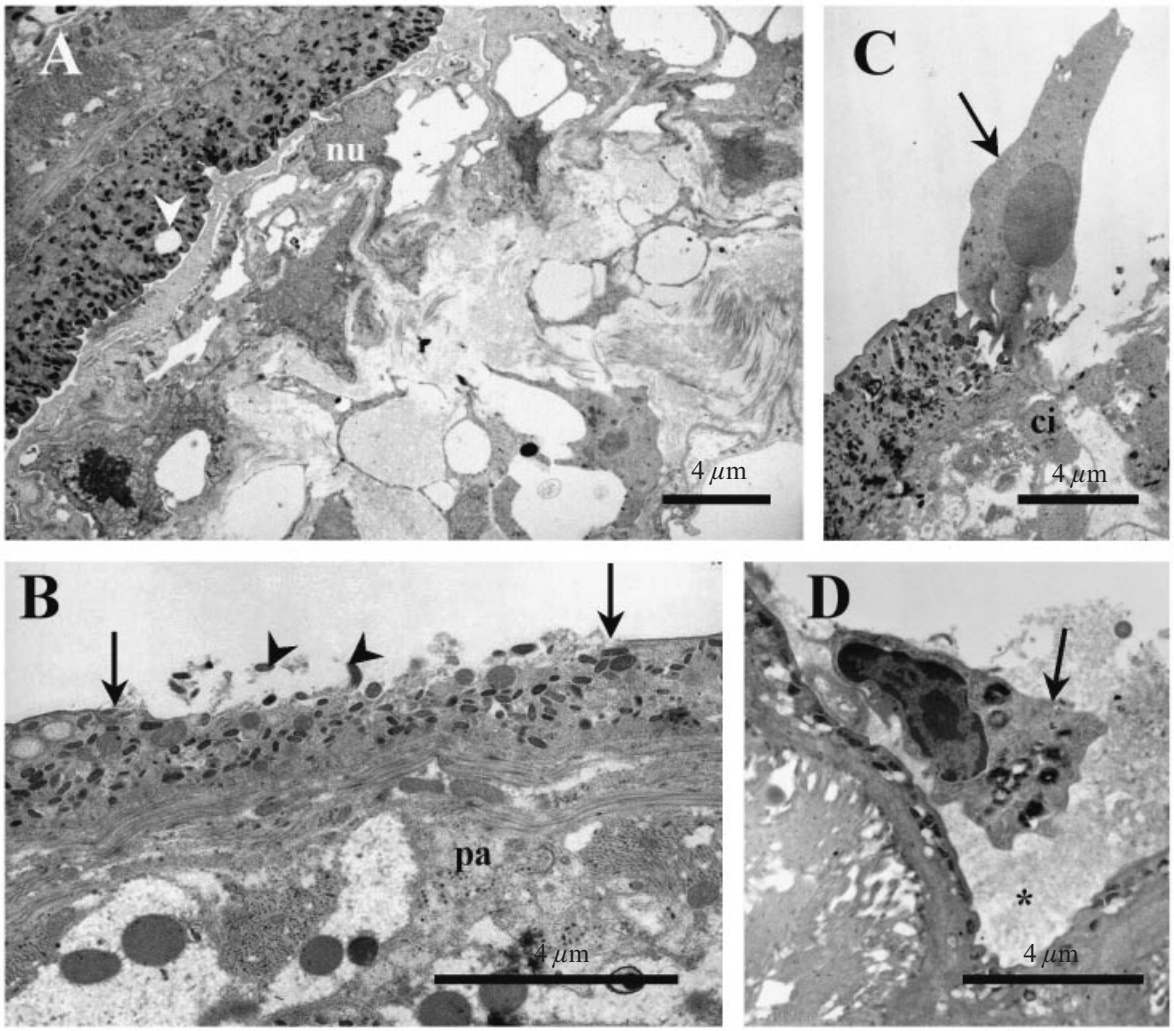

Fig. 5. *Pseudodiplorchis americanus* juveniles infecting alveolar epithelium. TEM. (A) 23 days p.i. Parasite tegument intact with the exception of small vesicles (arrowhead) at infrequent intervals along the surface layer; prominent nucleus (nu) in host epithelium. (B) 23 days p.i. Breach in the plasma membrane of the parasite tegumental surface layer (paired arrows); cytoplasm and tegumental vesicles (arrowheads) exposed to exterior. No indication of damage in underlying parenchyma (pa). (C) 44 days p.i. Part of tegumental surface layer stripped away exposing circular muscle blocks (ci); putative host white blood cell (arrow) intimately associated with parasite. (D) 44 days p.i. Putative host macrophage (arrow) contained numerous lysosomes in close contact with tegument of parasite's haptoral sucker; lung exudate (asterisk) within the cup formed by the parasite's sucker.

2A and B). Allowing for the limitations of local sampling of lung tissue by EM sectioning, there were no obvious differences in alveolar ultrastructure correlated with worm burden nor with infection duration. All hosts had carried their lung burdens for the normal period of respiratory infection before migration to the bladder. Pathogenic effects were comparable in hosts infected in Arizona and those maintained in the lab in the UK before re-infection.

Some areas of the infected lungs (e.g. Fig. 3A) were similar in appearance to the thin alveolar septa of animals uninfected in the year prior to examination, and fibrous tissue in the interstitium was observed (Figs 3 and 4). However, many regions (ca. 70% of the tissue examined by TEM), and all parts of the lung to which a parasite was still in contact, exhibited some form of abnormality. Vacuolation, associated with capillaries, interstitium, and particularly the epithelial cells, was common (Fig. 3B). Large vacuoles caused the epithelial cells to bulge into the alveolar air space, and/or microvilli were disrupted by blebs of the outer plasma membrane (Fig. 3C). Rarely, the plasma membrane was discontinuous, but interstitial oedema, distension of the epithelium and capillaries, and deposition of connective tissue appeared to result in thickening of the alveolar septa. Furthermore, epithelial cells in close proximity to the tegument of *P. americanus* were often extended into fine cytoplasmic processes (Fig. 3D).

Amorphous, electron-dense material, presumably composed of plasma protein, containing fibrin strands and disintegrated cell organelles, filled many alveolar pockets of the infected lungs (Fig. 4A and B). This exudate surrounded some of the parasites fixed *in situ* but was not observed actually bound to

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the tegument (Fig. 3D). Despite the fact that *P. americanus* is a blood-feeder, there was no obvious damage to the lung endothelium, but the abundance of alveolar exudate may reflect the leaky nature of the endothelium. Interstitial macrophages were commonly detected in infected lungs (e.g. Fig. 4B), identified by their large spherical nucleus with prominent nuclear envelope, homogeneous cytoplasm, and abundant vacuolar inclusions and lysosomes. Cells with similar morphology were also observed lying free in the lumen (Fig. 4C). Alveolar macrophages that normally adhere to the alveolar wall, or are immersed in the surfactant lining, typically become dislodged during routine histological processing, and may be found apparently floating free in the lumen (Burri, Gil & Weibel, 1994). However, it is not known whether the dendritic cells present in the lumen of infected lungs, often surrounded by exudate, were macrophages as they lacked the characteristic lysosomes. Macrophages/lymphocytes were found in the vacuoles underlying the epithelial cells in infected lungs only (Fig. 4D). No vacuoles, oedema or luminal exudates were identified in lung tissue from animals free of invasion for the preceding year.

Previous work describing the tegumental adaptations of juvenile *P. americanus* during migration through the gut has documented normal tegumental ultrastructure (Cable & Tinsley, 1992). This provides the basis for comparison with the current study. Abnormalities, in the form of vacuoles within the tegumental surface layer, were observed in 2 out of 10 lung parasites fixed *in situ* (e.g. Fig. 5A). In addition, 2 out of 5 worms that had been dislodged from lung tissue and serially sectioned exhibited more severe tegumental defects. In one of these, small vacuoles (up to 1 μm diameter) and multilamellate bodies (0·3–1 μm diameter) were present in the cytoplasm of the surface layer. More significantly, in the second parasite, breaches of the outer plasma membrane were evident extending over 1–5% of the body surface in transverse sections. Most of the underlying cytoplasm was intact but cytoplasmic strands and tegumental vesicles were dispersed at the surface (Fig. 5B). In one region (diameter 20 μm), the surface layer and basal lamina were completely stripped away exposing the underlying muscle, and a single lymphocyte/macrophage with dendritic processes was intimately associated with the parasite's tegument (Fig. 5C). Another detached pre-migrant was partially enclosed (over 10–25% of its surface area) by large aggregates of host alveolar exudate. Putative host white blood cells containing numerous lysosomes were embedded within this exudate (Fig. 5D) but the parasite's surface layer was undamaged.

DISCUSSION

The alveolar epithelium of the Amphibia is reported to be composed of either a single cell type or 2 different cells which resemble the Type I and II pneumocytes of mammals (reviewed by Welsch, 1981). Type I pneumocytes which overlie capillaries have flattened nuclei, bear numerous microvilli and contain few organelles, whereas more cuboidal Type II pneumocytes which are situated between capillaries, have irregular microvilli and contain numerous vesicles and multi-lamellate bodies. These inclusions are discharged into the alveolar air space where they contribute to the pulmonary surfactant layer at the epithelium–air interface (Burri *et al.* 1994). The single cell described in some amphibians (e.g. Meban, 1973) presumably fulfils the functions of the two kinds of alveolar cells in higher vertebrates (Welsch, 1981). All alveolar cells of *Scaphiopus couchii* contained vesicles and organelles but occasionally some cuboidal-like epithelial cells were observed which resembled cells depicted by Okada *et al.* (1962) in *Bufo vulgaris* and *Rana nigromaculata* as evidence of a second cell type (compare Fig. 1C above with Fig. 6 in Okada *et al.* 1962). However, similar cells in *Bufo icterus* were described as part of a monotypic epithelium (Berezin & da Silva Sasso, 1974). Without precise information on the position of these cuboidal cells in *S. couchii* they should not be assigned to a separate cell type. In higher vertebrates, Type II pneumocytes differentiate into Type I pneumocytes in response to damage to the alveolar lining (e.g. Wheater, Burkitt & Daniels, 1979; Richards, Masek & Brown, 1991). The epithelial cells of *S. couchii* in close contact with parasites showed evidence of increased metabolic activity, containing more vesicles and cell organelles, in addition to having larger rounded nuclei. Toads free of new infections in the year prior to EM study exhibited variable morphology of the alveolar epithelium. Approximately 5% of the alveolar lining was thickened, lacked capillaries and contained abundant interstitial fibrils. Prominent nuclei were more frequently observed in the overlying epithelium but there was no evidence of an entirely cuboidal epithelium that is characteristic of repaired alveolar tissue (Richards *et al.* 1991). Other regions of the lung epithelium of *S. couchii*, although not overlying thickened tissue, may have been affected by previous *P. americanus* infections. Toads guaranteed to be naïve with respect to *P. americanus* can not be obtained in field samples where invasion is a universal outcome of mating assemblies. Examination of adults reared from tadpoles in the laboratory would be necessary to clarify unambiguously whether the alveolar epithelium has a single or dimorphic cell structure.

The transmission dynamics of the *Pseudodiplorchis–Scaphiopus* system is unusual in that annual recruitment is typically more or less simultaneous, focused into a single pulse of mass invasion (occasionally 2 or 3) over less than 24 h/year. This

contrasts with the gradual trickle infection that characterizes most helminth/vertebrate interactions. It follows that laboratory experimental invasion induced by a single exposure to infective stages is exactly equivalent to that in the field: infection creates a sudden demand on host tissues and a single parasite cohort typically develops more or less synchronously through the life-cycle stages.

Although parasite-induced effects in the lungs are normally restricted to a few weeks, the duration of any pathological interactions may be extended when several transmission events affect a given host population. Tinsley & Jackson (1988) and Tocque (1993) documented seasons in Arizona in which 3 discrete host spawnings spanned a 3-week period, producing successive waves of mass invasion and doubling the period when the lungs would be subjected to *P. americanus* infection. In addition, small numbers of juvenile worms may remain in the lungs over winter: these undergo no further growth or development until they migrate from the lungs in the following summer but they remain active and continue to feed on blood (Tinsley & Jackson, 1986).

S. couchii can live for up to 17 years in the Sonoran desert (Tinsley, 1999) and, after sexual maturity, may be re-infected with *P. americanus* annually. The present study indicates that the relatively short-term pulmonary infection causes significant damage and the host may accumulate pathological effects. Vacuolation of respiratory epithelial cells is common, numbers of putative interstitial and alveolar macrophages are increased and exudate fills alveoli of infected lungs. The fibrous interstitial layers, associated with oedema and an increase of macrophages caused by acute infections, may also reflect residual damage from previous years' infections. Typically, the lung epithelium is susceptible to damage, not having the high repair capacity of the endothelium (Bachofen, Bachofen & Weibel, 1988).

Alveolar macrophages are observed rarely in normal amphibian lung, although macrophage-like cells are sometimes found in the connective tissue underlying the alveolar epithelium (Welsch, 1981). Experimental administration of foreign particles induces the appearance of amphibian alveolar macrophages that are either derived directly from blood leucocytes (as occurs in mammals) or migrate from adjacent connective tissue (Welsch, 1983). The alveolar epithelium is also capable of phagocytosis, so these cells have a dual secretory and lysosomal function. In this study, there was no evidence of any immune cells in the lungs of *S. couchii* uninfected for 1 year. However, immune cells were frequently observed in parasitized lung tissue, lying free in the alveolar space or within the epithelium or interstitium. These cells cannot be identified reliably from morphology alone (Weibel & Crystal, 1997) and are referred to here as leucocytes or macrophage-like-cells.

In association with the pronounced damage to the lungs and increased abundance of immune cells in infected *S. couchii*, damage to parasites was also recorded. Small vacuoles in the tegumental surface layer have previously been observed only in juveniles transferred experimentally to adverse conditions including gut digestive fluids (see Fig. 5B in Cable & Tinsley, 1992). A macrophage-like cell was observed attached to a parasite and the adjacent tegumental surface layer had been stripped away. This parallels the findings of Hoole & Mitchell (1984) on damage to the tegument of *Gorgoderina vitelliloba* by leucocytes in the kidneys of its frog host. The mechanisms involved in host responses against monogeneans are incompletely known (Buchmann, 1999). Studies on other helminths suggest the importance of host leucocytes (e.g. Morley & Hoole, 1995; Richards *et al*. 1996; Sharp, Pike & Secombes, 1991), and these have been shown to bind to the ectoparasitic monogenean, *Gyrodactylus derjavini* (see Buchmann & Bresciani, 1999).

The observed pathology has the potential to affect host respiration via interference with oxygen delivery. Heavy infections of the lung nematode *Rhabdias bufonis* negatively affect growth, locomotory performance and survival of *Rana sylvatica* and *Bufo bufo* (Goater & Ward, 1992; Goater & Vandenbos, 1997). In *S. couchii*, effects of lung pathology on respiratory efficiency could have greatest significance during the summer activity season when the toads emerge to feed. In this period, only about 20 nights per year (Tinsley, 1999), they must accumulate resources for growth and reproductive investment and store lipid reserves sufficient for the following 10–11 months of total starvation. If pulmonary damage, experienced during this same period, should compromise energetic expenditure and prey capture, then the ability of infected toads to survive hibernation could be prejudiced. *S. couchii* has an additional major energy demand essential for survival: excavation of burrows in the desert soil requires considerable effort at the end of each nocturnal foraging period and, critically, the toads must dig deep burrows prior to hibernation.

It is a significant feature of the *Pseudodiplorchis–Scaphiopus* interaction that the 3–4 week pulmonary phase coincides with highest environmental temperatures. When inactive during July/August, the toads bury themselves about 5 cm below the desert surface: field records at this depth indicate that the toads experience mean daytime temperatures around 34 °C, falling at night to around 22 °C (Tocque & Tinsley, 1991). Elevated temperature influences the rate of parasite feeding: blood intake of juvenile *P. americanus* doubles with a 5 °C increase (Tocque & Tinsley, 1992). Not only will the rate of host blood loss (and hence parasite growth and development) be greatest at these high temperatures, but also the temperature-dependent host immune

response will be most efficient. Tocque & Tinsley (1994*b*) demonstrated that if infected *S. couchii* are maintained in the laboratory at 25 °C, all parasites are lost within 11 months (whereas at 15–20 °C, there is no significant decline in burdens for over 14 months). Thus, the short-term lung infection in the *P. americanus* life-cycle is likely to represent a highly dynamic interaction between parasite feeding and development – and hence pathogenic damage to the host – and immune-mediated parasite mortality.

Data on the population dynamics of *P. americanus* provide evidence for powerful regulation of infection levels. Only about 3 % of the larvae successfully invading the host population survive until the first opportunity for reproduction. Tinsley (1999) reviewed circumstantial evidence from field and laboratory studies for involvement of host immunity in this mortality. While there is no direct confirmation of this hypothesis for *P. americanus*, parallel studies on the monogenean *Protopolystoma xenopodis* in *Xenopus laevis* have demonstrated a very effective immune response (Jackson & Tinsley, 2001). Field data on the population biology of *P. xenopodis* and *P. americanus* are interpreted in terms of acquired immunity that protects against accumulation of parasite burdens despite repeated re-infection (Tinsley & Jackson, 2002). Thus, following the virtual saturation of the *S. couchii* mating populations (100 % prevalence, mean intensity 100 worms/host), at least half of infected toads lose their parasites entirely and, in the remainder, mean intensity falls to 6 worms/host. Tocque & Tinsley (1994*b*) suggested that a major part of this attrition occurs in the lungs. The present study provides the first direct visualization of putative defensive responses, including destruction of parasite tegument by host macrophage-like cells.

The EM demonstration of scarring that develops during the brief lung infection provides important insight into longer-term consequences for the host–parasite interaction. This epithelial damage includes a reduction in capillary supply, potentially reducing respiratory efficiency, and was evident in toads free of lung invasion for 1 year.

The negative effects in this host–parasite interaction are pre-determined by different aspects of host biology. Initial pulmonary worm burdens are strongly influenced by host behaviour. Larval invasion is correlated with activity in mating assemblies, especially the frequency and duration of immersion of *S. couchii* in water contaminated with oncomiracidia. Thus, females (usually entering water only once for a few hours to spawn) tend to acquire lower lung burdens than males, and males unsuccessful in mating (that continue to search through relatively greater volumes of water) tend to acquire higher burdens than males that mate (and are more sedentary during spawning) (Tinsley, 1989). Host behaviour may therefore be a significant determinant of acute pathology. In contrast, the extent of chronic pathology, the removal of host blood during hibernation, is likely to be determined primarily by immune competence. Host fitness with respect to ability to limit infection levels would be maximal in those individuals (generally 50 % of the population) that eliminate their worm burdens entirely, removing the costs of chronic infection. It is highly significant, therefore, that scarring resulting from an acute pulmonary infection may still create a chronic cost for the host, even for those strongly immunologically competent hosts that can eliminate established parasites. So, the overall pathogenic interaction in the *Pseudodiplorchis–Scaphiopus* system has 2 consequences: some host individuals may suffer a continuous drain on resources caused by adult worm burdens that may be carried for successive years. However, toads that are parasite-free by virtue of their ability to reject re-infection may also carry a cost – in terms of lung scarring – from the 'ghosts of infections past'.

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