Rostellar hook morphology of *Echinococcus granulosus* (Batsch, 1786) from natural and experimental Australian hosts, and its implications for strain recognition

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SUMMARY

An analysis of the rostellar hooks of Australian isolates of *Echinococcus granulosus* revealed that there was less variation in larval (metacestode) than adult characters and that metacestode characters could be measured directly from adult worms. A factor analysis indicated that two factors, one representing a contrast between number of hooks and their length, and the other representing blade lengths, were sufficient to account for 87·5% of the variance in metacestode hook measurements. These results indicate that rostellar hook morphology is not useful for discriminating strains of *E. granulosus* in Australia. The Tasmanian and mainland domestic strains were found to be indistinguishable using rostellar morphology. Although many of the isolates from sylvatic hosts differed from those from domestic hosts, there was not a clear separation as would be expected if two distinct strains existed. Evidence was presented to show that the morphological differences seen in sylvatic hosts could be attributed to host-induced effects, and that the previously accepted existence of two mainland strains should be investigated further.

Key words: *Echinococcus granulosus*, rostellar hook morphology, factor analysis.

INTRODUCTION

Three distinct strains of *Echinococcus granulosus* have been described in Australia (Kumaratilake, Thompson & Dunsmore, 1983; Kumaratilake & Thompson, 1982, 1983). One of these is restricted to the island state of Tasmania, while the other two have been found in most parts of mainland Australia, but not in Tasmania. Life-cycles of the two mainland strains are thought to be restricted predominantly to either domestic or sylvatic hosts, and are known as the domestic sheep strain and the Australian sylvatic strain (Kumaratilake & Thompson, 1982). Transmission of the domestic strain to sylvatic hosts has been reported (Thompson & Kumaratilake, 1985).

Isolates of *E. granulosus* from throughout Australia are routinely sent to this laboratory for strain-typing. It is considered that humans are susceptible to infection with all Australian strains (Baldock, Thompson & Kumaratilake, 1985; Thompson *et al.* 1987). Differentiation of the two mainland strains has been considered to be important epidemiologically in order to determine cycles of transmission. This is particularly important for human cases so that the origin of infection may be quickly identified.

One accepted method of distinguishing Australian strains has been to use the morphological criteria proposed by Kumaratilake & Thompson (1984*a*). Differences in rostellar hook morphology have been thought to provide the quickest and most reliable means of differentiating the domestic and sylvatic strains on the mainland. Rostellar characters which have been used are the number and arrangement of hooks, and the total length and blade length of large and small hooks from both adult worms and protoscoleces. Differences in strobilar morphology between the mainland strains in 35-day-old worms from experimental infections in dogs also have been observed (Kumaratilake & Thompson, 1984*a*). Useful characters are considered to be total worm length, position of the genital pore, number of testes and the anatomy of the female reproductive duct system.

This paper reports on the suitability for strain-typing of the established rostellar hook characters. The relative importance and variability of a number of rostellar hook characters is determined. The difficulty in allocating a number of isolates to existing strains is discussed, as is the status of Australian strains.

MATERIALS AND METHODS

The isolates used in this study were obtained from either the reference collection of the WHO Collaborating Centre for Echinococcosis/Hydatidosis at Murdoch University, or were sent to this laboratory as viable hydatid samples. During the course of this study, Lymbery & Thompson (1990) demonstrated the existence of genetic differences between cysts within a host individual. However, for the purposes

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Table 1. Details of geographic and host origin, and sample sizes of isolates used in this study

Isolates used in factor analysis

Location	Host: Macropod marsupials	Sheep	Cattle	Pigs (feral)
Eastern mainland Australia	20	22	1	5
Western Australia	9	7	1	1
Tasmania		4	2	—

Additional naturally infected isolates

Location	Host	Number
Eastern mainland Australia	Human	5
Eastern mainland Australia (Zoo)	Monkey	2
Tasmania	Human	1
Tasmania	Wombat	1
United Kingdom	Horse	2
USSR	Sheep	1
Egypt	Camel	1

Experimentally infected isolates

Source location	Source host	Recipient host	Number
Queensland	Cattle	Rat	1
Queensland	Pig	Mouse	1
Queensland	Pig	Rat	1
Eastern Australia	Sheep	Rat	1
New South Wales	Sheep	Rat	1
Tasmania	Sheep	Macropod	1
Western Australia	Sheep	Rat	2

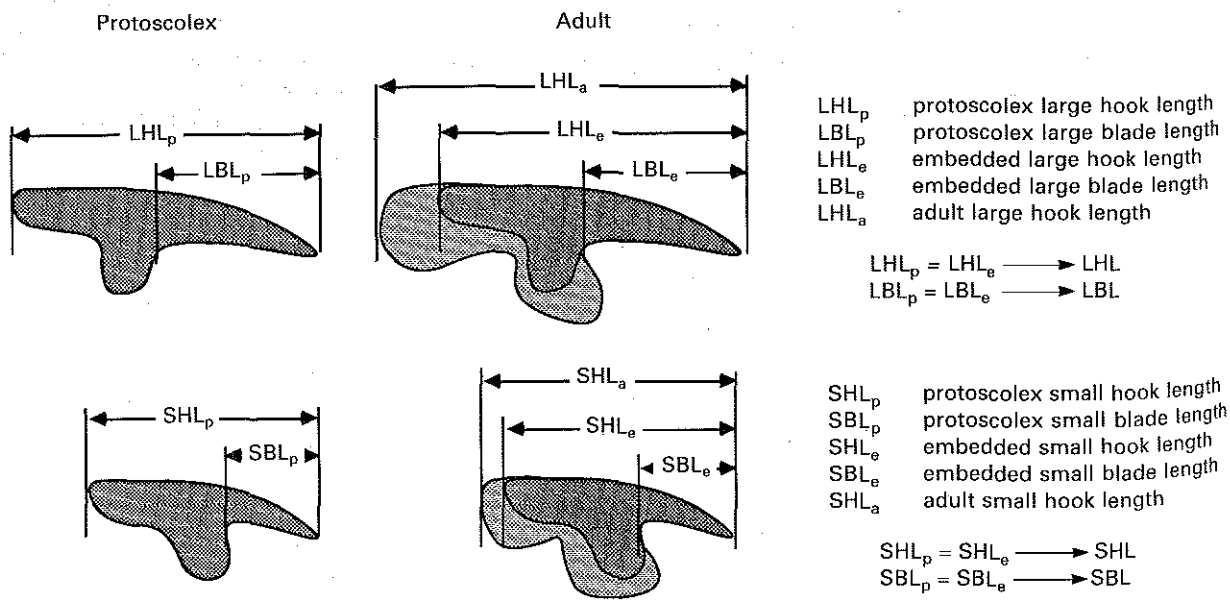

Fig. 1. Diagrammatic representation of hooks of *Echinococcus granulosus* showing measurements taken in this study.

of this study, an isolate was considered as one or several hydatid cysts from an individual host. The geographic and host origin of the isolates used is summarized in Table 1. Isolates from the reference collection had been treated as described by Kumaratilake & Thompson (1983, 1984*a*). Living protoscoleces were removed from hydatid cysts obtained from various Australian localities. Either unfixed, or fixed (10% formalin) protoscoleces were squashed under cover-slips in polyvinyl lactophenol on microscope slides. Three large and 3 small hooks were measured, and all the hooks were counted from each of 6 protoscoleces from each isolate. Lubinsky (1960) counted only those hooks which were part of a large and small pair, but in this study we have counted all the hooks in order to avoid subjectivity. This method was followed also by Sweatman & Williams (1963), and Kumaratilake & Thompson (1984*a*). Measurements taken were LHL_p, LBL_p, SHL_p and SBL_p (Fig. 1). The blade length measurements conform to those of Sweatman & Williams (1963) and Kumaratilake & Thompson (1984*a*), which differ from those of Rausch (1953). In some cases, protoscoleces were fed to dogs and adult worms recovered after 35 days. The same number of scoleces from a sample of these worms was measured and counted as indicated above for protoscoleces. Measurements taken were LHL_a, LHL_e, LBL_e, SHL_a, SHL_e, and SBL_e (Fig. 1).

The sample sizes were chosen for logistic reasons. Since the aim was to be able to use the measuring system for quick diagnosis of an isolate as one or other of the strains, it was felt necessary to use relatively small numbers. Subsequent nested analyses of variance showed that most of the variance was between isolates, indicating that the measurement of more hooks would have been redundant.

Standard statistical procedures referred to below are from Zar (1984). The factor analysis used was the principle components method in StatView 512+™ (Abacas Concepts, Inc.). The criterion used for determining the number of factors to extract was the greater of the numbers determined by the 75% variance rule and the root curve criterion of Cattell (1966).

RESULTS

The growth of hooks in the definitive host is apparently achieved by the addition of hook material which is different from that laid down in the protoscolex. This material is added only to certain parts of the hook, and the blade in particular appears to be unchanged from the metacestode to the adult stage. The protoscolex hook can be seen clearly to be a part of the adult hook (Fig. 2). In order to show that the embedded hook measurements taken on a sample of adult hooks were equivalent to the measurements taken from a sample of protoscolex hooks, factorial analyses of variance were performed. All 11 of the isolates for which both protoscolex hook and blade lengths and embedded hook and blade lengths were measured, were included. Whereas there were highly significant differences between isolates for all 4 variables, there was only one small but significant difference between stages (Table 2). However, in two cases there was a significant interaction between factors, indicating that although overall means were not different, within some isolates there were significant differences between the stages. Significant interaction effects were further analysed by least significant differences at the 1% level. This conservative level was chosen because it was considered that 11 pairs of means was too many for the 5% level to be used. Only 1, and 2 isolates respectively were found to have significantly different means between the stages, for large hook length and large blade length. We interpret these results as an indication that measurements taken from either the larval or adult stage may normally be treated as equivalent. Consequently, all of the hook measurements previously accepted as standard (Sweatman & Williams, 1963; Kumaratilake & Thompson, 1984*a*) may be measured from adult hooks. Thus, the blade length of a large hook as measured from protoscoleces (LBL_p) can be regarded as equivalent to the embedded blade length measured from adult hooks (LBL_e). Hereinafter we shall use the code LBL for this measurement. Similarly, the other measurements will be referred to as LHL, SHL and SBL.

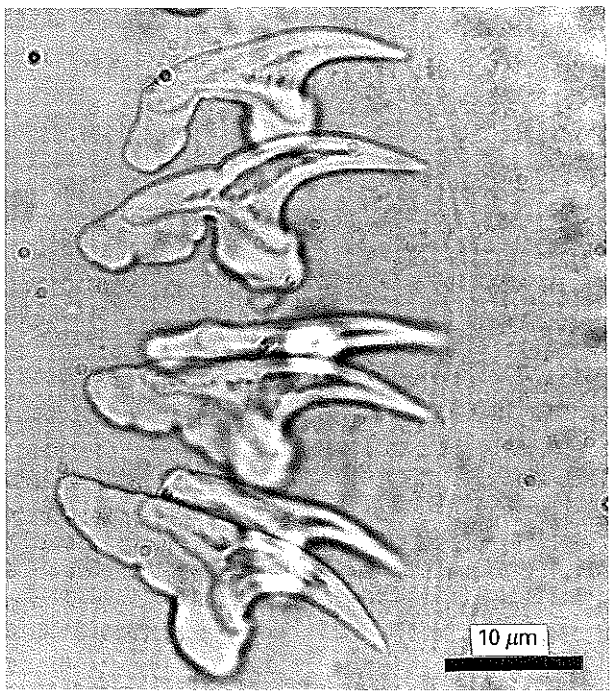

Fig. 2. Photograph of several rostellar hooks from a 35-day-old adult *Echinococcus granulosus*. Note that entire metacestode hooks can be seen clearly within the outline of adult hooks.

Table 2. Results of 2 factor analysis of variance (isolates and stages)

Source	D.F.	MS	*F*	Stage means (μm) Protoscolex	Adult
		Length of large hook (LHL)			
Isolate	10	53·793	41·52***	25·0	24·8
Stage	1	4·245	3·28		
Isolate × stage	10	2·789	2·15*		
Error	374	1·295			
		Length of large blade (LBL)			
Isolate	10	6·775	15·05***	13·0	13·1
Stage	1	0·023	< 1		
Isolate × stage	10	1·551	3·44***		
Error	374	0·450			
		Length of small hook (SHL)			
Isolate	10	97·319	40·63***	20·9	21·2
Stage	1	14·938	6·24*		
Isolate × stage	10	0·981	< 1		
Error	372	2·395			
		Length of small blade (SBL)			
Isolate	10	7·507	11·02***	9·1	9·2
Stage	1	2·263	3·32		
Isolate × stage	10	0·682	1·00		
Error	371	0·681			

* $P < 0{\cdot}05$ *** $P < 0.001$

Table 3. Mean coefficients of variation (%) for adult total length (LHL_a and SHL_a), and protoscolex total length (LHL_p and SHL_p)

	Protoscolex	Adult	*t* test	D.F.	Significance
Large hook	4·2	5·1	2·45	12	$P < 0{\cdot}05$
Small hook	6·6	9·4	4·74	11	$P < 0{\cdot}001$

There were 21 isolates for which both the protoscolex or embedded hook lengths (LHL and SHL) and adult hook lengths (LHL_a and SHL_a) were measured. Although there were highly significant correlations ($P < 0{\cdot}001$) between these variables ($r = 0{\cdot}66$ and $r = 0{\cdot}94$ for large hooks and small hooks respectively), the coefficients of variation of adult hook lengths were found to be significantly greater than that of protoscolex hook lengths for both large and small hooks (Table 3). There were many isolates for which adult hook length was not available, whereas protoscolex hook lengths were available for all isolates. It was decided in view of the high correlation with protoscolex lengths, and the higher variability, not to use the adult hook lengths in the factor analysis, so that a greater number of isolates could be included.

Factor analysis was carried out on the 5 remaining variables in 72 isolates from macropod marsupials, cattle, sheep and pigs from Western Australia, eastern mainland Australia, and Tasmania. Although the pig isolates were from feral hosts, sheep, cattle and pigs will be called domestic hosts below. There were significant correlations between most of the variables (Table 4). All of the hook lengths were positively correlated with each other. The number of hooks was negatively correlated with the length of both the large and small hooks, but there was not a significant correlation between number of hooks and either LBL or SBL.

In the factor analysis, two factors were extracted accounting for 87·5 % of the total variance in measured rostellar hook characters. The first factor accounted for 58·9 % of the variance and is primarily a contrast of number of hooks, and the length of both large and small hooks. Factor 2, explaining 28·6 % of the total variance, is mainly due to the blade lengths of the large and small hooks. Intercorrelation between the two factors is 0·35. Factor loadings are shown in Table 5. Scores for the two factors were computed for each isolate and plotted against each other. Tasmanian isolates were morphologically indistinguishable from mainland domestic isolates (Fig. 3). Isolates from macropod hosts were generally

Table 4. Correlation matrix for 5 rostellar hook measurements using 72 isolates from mainland Australia and Tasmania

(Character codes are as follows: number of hooks (NH), large hook length (LHL), large blade length (LBL), small hook length (SHL) and small blade length (SBL).)

LHL	−0·58***			
LBL	−0·03	0·63***		
SHL	−0·74***	0·85***	0·38***	
SBL	−0·02	0·38**	0·72***	0·37***
	NH	LHL	LBL	SHL

** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

Table 5. Oblique solution factor loadings for 5 rostellar hook measurements using 72 isolates from mainland Australia and Tasmania

(Character codes are as follows: number of hooks (NH), large hook length (LHL), large blade length (LBL), small hook length (SHL) and small blade length (SBL).)

	Factor 1	Factor 2
NH	−1·006	0·341
LHL	0·741	0·369
LBL	0·004	0·937
SHL	0·892	0·155
SBL	−0·118	0·938

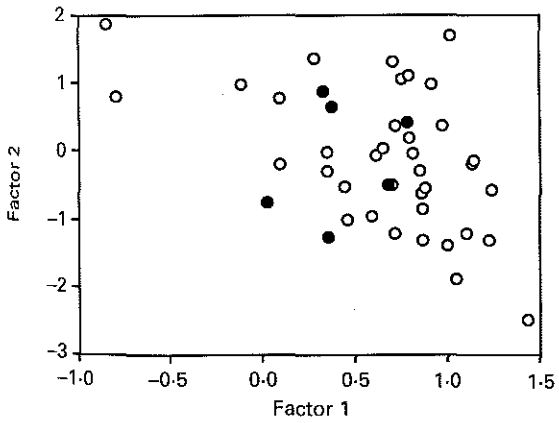

Fig. 3. Scatterplot of scores for the two factors extracted by factor analysis, for isolates of *Echinococcus granulosus* from 43 naturally infected domestic Australian hosts. (○) Mainland; (●) Tasmania.

found to differ from isolates from domestic hosts, particularly in Factor 1, although there was considerable overlap (Fig. 4). Factor 2 does not appear to be useful in discriminating between groups of isolates.

Since Factor 1 is the contrast of number of hooks (NH) with size of hooks, and is the only axis of separation between wild and domestic isolates, a plot of LHL against NH was drawn for the 72 isolates used in the factor analysis, plus additional isolates

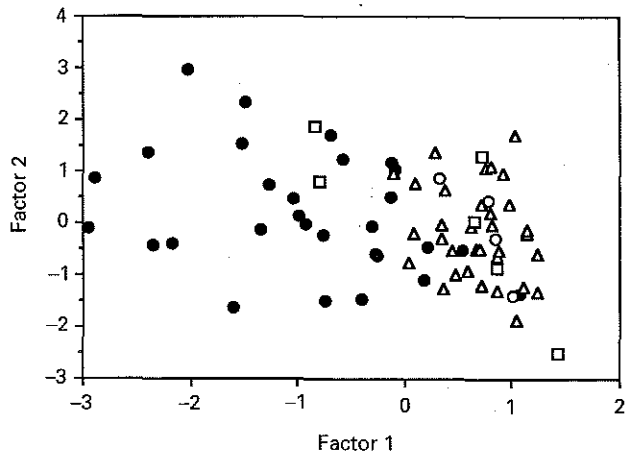

Fig. 4. Scatterplot of scores for the two factors extracted by factor analysis, for isolates of *Echinococcus granulosus* from 72 naturally infected Australian hosts. (●) Macropods; (△) sheep; (○) cattle; (□) feral pigs.

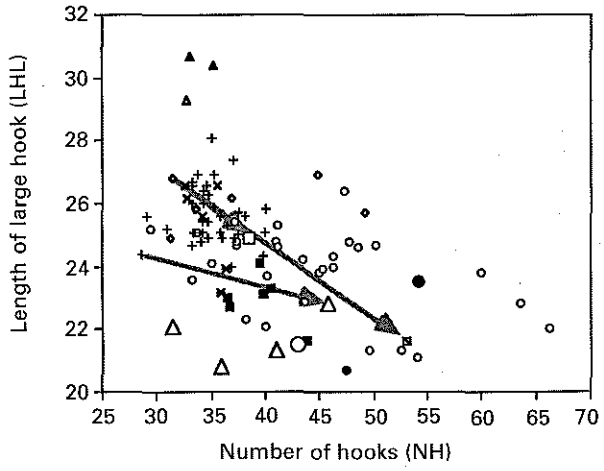

Fig. 5. Scatterplot of the length of the large hook (LHL) and the number of hooks (NH) for all isolates of *Echinococcus granulosus* measured in this study. Where the source material for passaged isolates was measured, an arrow connects the points for the source and passaged material, with the tip of the arrow at the point for material grown in the recipient host. (○) Macropod; (+) sheep; (×) cattle; (◇) pig; (■) human; (✖) monkey; (▲) horse; (△) camel; (●) wombat; (●) sheep to macropod; (△) sheep to rat; (□) pig to rat; (○) cattle to rat; (◘) pig to mouse.

from other hosts (Fig. 5). Two isolates of UK horse origin and 1 from an Egyptian camel are quite distinct from the Australian isolates, whereas 1 sheep isolate from Russia is indistinguishable. The Australian isolates fall roughly into 2, not discontinuous groups. Sheep isolates form a relatively discrete group with a small number of large-sized hooks, whereas the macropod isolates were far more varied, some being similar to the sheep isolates, and others with a large number of smaller sized hooks.

The isolates which were passaged from their original intermediate host to laboratory rats and mice are also shown in Fig. 5. Those for which the

original isolate was measured are connected to the original by an arrow. These data show a large shift in rostellar hook morphology for both hook size and number of hooks. One Tasmanian sheep isolate which was passaged to a red-necked wallaby (*Macropus rufogriseus*) as a secondary cyst, has an appearance more typical of other macropod isolates than sheep isolates. Six human isolates also are included in Fig. 5. All of these have relatively small hooks and are somewhat intermediate between domestic and macropod isolates in hook number. They do not clearly belong to either of the main groups, but are most similar to isolates passaged to mice and rats. Two isolates from macaque monkeys from the Adelaide Zoological Gardens are similar to the human isolates in both hook number and size.

In summary, the results have indicated firstly that protoscolex hook characters are all measurable from adult hooks. Adult total hook lengths are more variable than protoscolex hook lengths with which they are highly correlated. The factor analysis on number of hooks, and lengths and blade lengths of the large and small hooks showed that these 5 variables are correlated with each other in such a way that they may be explained by just two factors. Only one of these factors [the contrast between number of hooks (NH) and total lengths of hooks (LHL and SHL)] appears to sort isolates by host, yet there is no clear distinction between domestic and sylvatic isolates for this factor. Isolates which had been passaged to other host species resulting in secondary cysts show that the rostellar morphology in the secondary host differs from that found in the original host.

DISCUSSION

The usefulness of morphological criteria as the sole means for the differentiation of some strains of *E. granulosus* has been brought into question recently (Thompson & Lymbery, 1988). However, for workers in the field of epidemiology there is the need for a quick method of strain identification, and at present there are no other means of rapid differentiation.

Strobilar characters are more numerous than rostellar characters but there are some disadvantages to their use. They are soft and subject to change by the effects of fixation. They also vary with the age of the worm, so that many strobilar characters are useful only in comparing worms from experimental infections of known age (Kumaratilake *et al.* 1983). Rostellar hooks are hard, and although there are fewer characters to be derived from them, these are not changed by fixation. The same characters are available in both adults and larvae, and are easy to measure, requiring less experience in interpretation.

Previous studies have treated measurements from larvae and adults as separate characters (e.g. Sweatman & Williams, 1963; Kumaratilake & Thompson, 1984*a*). Although it has been well known that hook growth in adults is only in the handle and guard (Cameron, 1926; Rausch, 1953), it has not been appreciated that the unchanged larval hook is embedded in the extra material laid down in the adult, and that its size and shape may be observed readily there. Yamashita, Ohbayashi & Konno (1956) demonstrated that growth of the handle and guard continues for up to a year in *E. granulosus*, which means that if the total length of the adult hooks is to be used as a character, the age of the worm must be taken into consideration. This is only possible for experimental infections. The shape of the adult hooks is highly variable (Sweatman & Williams, 1963), and we have found that although the size is correlated with size of the larval hook, it is significantly more variable.

There were some significant differences between the stage at which hook measurements were taken, either across all isolates (SHL), or within certain isolates (1 of 11 isolates for LHL and 2 of 11 for LBL). We believe that these differences merely reflect the low variation within individual protoscoleces. Dogs were usually infected with protoscoleces pooled from several cysts, whereas the sample of protoscoleces for hook measurements usually came from a single cyst. If individual cysts in the intermediate host are from heterogeneous sources (Lymbery & Thompson, 1990), it is possible that the adult worms sampled for measurement were genetically different from the protoscoleces, and this may explain the differences in hook measurements.

A number of rostellar characters have been used to differentiate species and strains of *Echinococcus* (Rausch, 1953; Rausch, Rausch & D'Alessandro, 1978; Sweatman & Williams, 1963; Kumaratilake, Thompson & Eckert, 1986; Eckert *et al.* 1989). Thompson & Lymbery (1988) pointed out the need to identify character redundancies, as the use of a large number of correlated characters may provide a false impression of phenotypic similarity. Gardner, Rausch & Camacho (1988) found relatively few correlations between hook dimensions in *Echinococcus vogeli*; however, it appears from the present study of Australian isolates of *E. granulosus* that all rostellar hook characters can be condensed to two functional characters.

Our results clearly show that Tasmanian and mainland domestic strains are indistinguishable by rostellar morphology. However, there is strong evidence to show that these two strains differ biologically, for example in the speed of development in the definitive host (Kumaratilake *et al.* 1983). Only one factor, the contrast of number of hooks with length of large and small hooks, was useful in differentiating isolates from domestic and sylvatic hosts. The unexpected result of the factor analysis was the lack of a clear separation into domestic and

sylvatic strains as has been reported in earlier studies (e.g. Kumaratilake & Thompson, 1984*a*). All sheep isolates had a small number of larger sized hooks, but there exists a gradation of macropod isolates from those resembling sheep isolates at one end of the spectrum, to those with a greater number of smaller sized hooks at the other end. Isolates which had been passaged from sheep to other intermediate hosts, such as rodents or a wallaby, no longer had the characteristic appearance of sheep isolates. This is suggestive of host-induced changes in both hook size and number.

There is ample evidence in the literature that the number and size of *Echinococcus* spp. hooks are subject to host-induced variation. Lubinsky (1960) found a high degree of variability between isolates and stressed the need for the examination of a large number of scoleces. He showed that *Echinococcus* in the human host has significantly more hooks than in other hosts. This observation is supported by our findings: all the human isolates in this study had smaller hooks, and most isolates had a greater number of hooks than those from normal domestic hosts. Vogel (1957) also noted that hooks of *E. granulosus* from humans were smaller than those from pigs. In a particularly detailed study, Sweatman & Williams (1963) managed to establish primary infections (i.e. using eggs) in a number of unusual intermediate hosts (2 mice, 6 rabbits, 2 possums, 1 wallaby and 1 cat). They showed that, for *E. granulosus* of New Zealand sheep origin, in unusual hosts there was an increase in number of hooks and a reduction in the length of both the large and small hooks. The form of *Echinococcus* in New Zealand sheep is thought to be identical with the domestic sheep strain on the Australian mainland (Kumaratilake *et al.* 1986). One isolate in our study had been passaged as a secondary cyst, from a Tasmanian sheep to a red-necked wallaby, resulting in the sylvatic appearance of rostellar hooks. This is particularly interesting because there has not been a report of a natural infection in Tasmanian macropods. These results suggest that the characteristic morphological appearance of the hooks of *E. granulosus* in Australian sylvatic hosts may be due to the host species rather than being indicative of an intrinsic sylvatic strain. If the characteristic morphology of the Australian sylvatic isolates is caused by the host species alone, we would predict that all the isolates from one host species would be morphologically similar to each other, but different from those from another host species. Fig. 6 shows 33 isolates from marsupials and feral pigs. The results are difficult to interpret due to small sample sizes, but some conclusions may be drawn from them. It is apparent that there are differences between species of marsupial hosts. For example, western and eastern grey kangaroo (*Macropus fuliginosus* and *M. giganteus*) isolates generally have relatively low

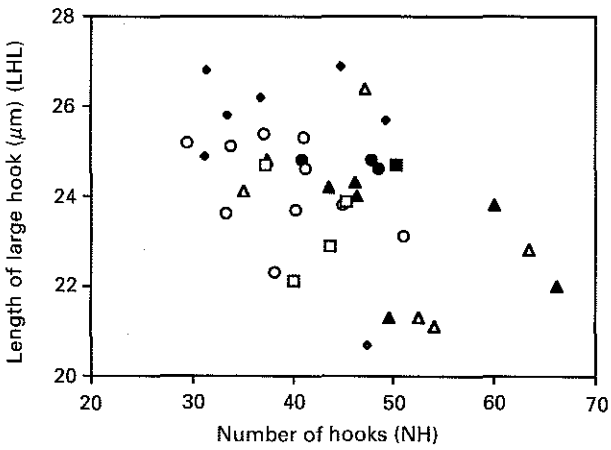

Fig. 6. Scatterplot of the length of the large hook (LHL) and number of hooks (NH) for isolates from naturally infected wild and feral Australian hosts. (▲) *Wallabia bicolor*; (●) *Macropus dorsalis*; (△) *M. rufogriseus*; (○) *M. fuliginosus*; (□) M. giganteus; (■) petrogale; (◇) wombat; (◆) feral pig.

numbers of large-sized hooks, whereas isolates from swamp wallabies (*Wallabia bicolor*) and red-necked wallabies (*Macropus rufogriseus*) have more but smaller hooks. However, some isolates do not fit this pattern, indicating that the effect of the host on rostellar morphology is not absolute and that other factors must be operating.

An alternative hypothesis to host-induced changes is that there are genetic differences behind the characteristic morphological appearance of domestic and sylvatic isolates, but that introgression occurs where the cycles overlap, leading to hybrid forms. These explanations are not mutually exclusive. In any event, our results are certainly at variance with the belief that the Australian mainland sylvatic and domestic strains are completely separate independently evolving populations, as previously suggested (Thompson & Kumaratilake, 1985).

Previous studies have reported differences between the Australian domestic and sylvatic strains in characters other than rostellar morphology. Table 6 summarizes these differences. We have been unable to find consistent differences in the shape of the adult hooks, testes number or the morphology of the female genital duct between a small number of isolates from domestic and sylvatic hosts, but further more systematic studies are required on these characters. Thompson & Kumaratilake (1985) reported differences in development between the strains in dingoes and domestic dogs. However, a limitation of such *in vivo* comparative developmental studies is the number of animals which can be used, and further investigations should be undertaken to fully substantiate these observations. That the sylvatic strain has never been observed in sheep has been used as evidence that sheep are refractory to infection with it, whereas the presence of both

Table 6. Summary of differences between the Australian mainland strains as reported in the literature

Criterion	Domestic	Sylvatic	Source*
Strobilar morphology of 35-day-old worms			
Number of testes	35	45	3
Female ducts	Loops absent	Loops present in oviduct, common duct, or seminal duct	3
Position of gonopore in sexually mature segment	Posterior (majority), or at midpoint	Slightly anterior (majority), or at midpoint	3
Position of sexually mature segment	Penultimate (majority), or terminal	Terminal (majority), or penultimate	3
Comparative development			
Dogs and dingoes	—	Develops more quickly in dingoes than in dogs	5
Sheep	Normal	Considered to be refractory	3
Secondary cysts in mice	Fair development	Poor	2
Isoelectric focusing	Different protein banding patterns		1,4

* 1, Baldock, Thompson & Kumaratilake (1985); 2, Kumaratilake & Thompson (1983); 3, Kumaratilake & Thompson (1984*a*); 4, Kumaratilake & Thompson (1984*b*); 5, Thompson & Kumaratilake (1985).

domestic and sylvatic rostellar morphology in macropods suggested their susceptibility to both strains (Kumaratilake & Thompson, 1984*a*). However, if the typical rostellar morphology in macropods is host induced, then the absence of the trait in domestic hosts cannot be taken as evidence of refractoriness. Experimental evidence is needed to clear up this question and a cross-infection trial is at present under way in our laboratory, and another Austalian laboratory (D. J. Jenkins, personal communication).

The remaining evidence from isoelectric focusing of soluble proteins (Kumaratilake & Thompson, 1984*b*) provides the strongest suggestion of a genetic difference between the strains. However, the complex patterns produced by the technique cannot be interpreted genetically with any confidence and it is possible that differences in banding patterns reflect host-induced changes in the expression of structural proteins, perhaps as a consequence of biochemical flexibility. A change in environment from one host species to another may impose constraints on metabolism which lead to altered biochemical pathways. Such metabolic flexibility in helminths has been proposed by Bryant & Flockhart (1986) and in a preliminary study, protoscoleces of *E. granulosus* of Australian sheep and wallaby origin differed significantly in metabolic end-products assayed *in vitro* (C. A. Behm, C. Bryant & R. C. A. Thompson, cited by Bryant & Flockhart, 1986).

In conclusion, whereas there is good evidence for a separate, though morphologically similar Tasmanian domestic strain, the evidence for a genetically different sylvatic strain of *E. granulosus* on the mainland of Australia is equivocal. Isoenzyme analysis, a more readily interpretable measure of genetic variation than isoelectric focusing of total proteins, has not supported the ideal of major genetic differences between Australian mainland populations (Lymbery, Thompson & Hobbs, 1990). The present study has found no clear morphological separation between isolates from domestic and sylvatic hosts. Rostellar morphology, like metabolism, may be a phenotypically plastic character, capable of host-induced variation. To resolve the conflict between this interpretation and that proposed by Kumaratilake & Thompson (1983, 1984*a*, *b*), and Thompson & Kumaratilake (1985), further studies on cross-transmission and on genetic differences in natural populations are required.

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