Genetic diversity and genetic differentiation in *Echinococcus granulosus* (Batsch, 1786) from domestic and sylvatic hosts on the mainland of Australia

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SUMMARY

Enzyme electrophoresis was used to examine genetic variation within and between populations of *Echinococcus granulosus* from domestic and sylvatic hosts in western and eastern Australia. Substantial genetic diversity was found within all populations. There was no evidence, however, of genetic differentiation between populations from different hosts or geographic areas. When isolates were grouped into previously described domestic or sylvatic strains on the basis of rostellar hook morphology, most (94 %) of the genetic variation occurred within, rather than between strains. These results conflict with the currently accepted theory of separate domestic and sylvatic strains of *E. granulosus* on the mainland of Australia.

Key words: *Echinococcus granulosus*, strains, enzyme electrophoresis, genetic diversity, genetic differentiation.

INTRODUCTION

Echinococcus granulosus occurs on every inhabited continent. Although the definitive host is almost always a canid, a very wide range of intermediate hosts are utilized. Many intraspecific variants, or strains of *E. granulosus* have been described from different geographic areas or intermediate host species (Kumaratilake & Thompson, 1982*a*; McManus & Smyth, 1986; Thompson & Lymbery, 1988). These strains usually differ in a number of biological characteristics, often of importance to the epidemiology and control of hydatid disease.

Two models have been proposed to account for strain variation in *E. granulosus*. Smyth & Smyth (1964) suggested that strains arise from mutations in obligately self-fertilizing adults, expressed in homozygous form by the zygote and amplified by asexual reproduction in the cystic stage. Alternatively, Rausch (1967, 1985) suggested that adults are normally cross-fertilizing and that strains arise, not as a direct consequence of the breeding system of *E. granulosus*, but as a result of geographic and ecological barriers between populations associated with different domestic hosts. These models are not mutually exclusive, and a recent electrophoretic study found a pattern of genetic variation within Australian strains indicating that both cross- and self-fertilization occur in natural populations (Lymbery & Thompson, 1988).

The existence of genetic diversity within populations may pose a problem in routine strain-typing studies, which often assume that all parasites in a particular species of host in a particular locality are genetically and phenotypically identical (Lymbery & Thompson, 1990). A more fundamental problem, however, arises from the likelihood that in such a wide-ranging species as *E. granulosus*, diversity within populations will be converted, by genetic drift or adaptive change, to differences in gene and genotype frequencies between populations. Should these populations be regarded as different strains? Thompson & Lymbery (1988) argued that the term 'strain' should be used as a practical descriptor denoting populations which differ genetically in characters of significance to the epidemiology and control of hydatid disease. The question of what constitutes a strain then becomes one of deciding which genetically differentiated populations are likely also to differ in characters of biological significance. As a first step, this requires a decision about where the major components of genetic variation lie. For example, is most variation found within local populations or between them? Is more variation found between populations from different species of host or between populations from the same host species in different localities?

On the mainland of Australia, *E. granulosus* is believed to be maintained in two different cycles of transmission: a domestic cycle principally between sheep and dogs, and a sylvatic cycle involving mainly macropod marsupials and dingoes (Kumaratilake & Thompson, 1982*b*). Evidence of morphological, biochemical and developmental differences between isolates of domestic and sylvatic origin led to their proposed designation as distinct strains (Thompson

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Table 1. Populations of *Echinococcus granulosus* from different geographic areas and hosts in Australia, which were sampled in the present study

Population code	Geographic origin	Host origin	Sample size*
EAS	Eastern Australia	Sheep	28
EAM	Eastern Australia	Macropods†	8
WAS	Western Australia	Sheep	12
WAM	Western Australia	Macropods‡	17

* Number of isolates, where an isolate refers to the protoscoleces and adults derived from 1 cyst.
† *Macropus giganteus* (1), *Wallabia bicolor* (6), *W. rufogrisea* (1).
‡ *Macropus fuliginosus*.

& Kumaratilake, 1985). Recent morphological studies, however, have demonstrated more variation within, and more overlap between isolates from domestic and sylvatic hosts than previously suspected (Hobbs, Lymbery & Thompson, 1990). The present study was designed to complement this morphological analysis by examining genetic variation between isolates from different host species and geographic areas in Australia.

MATERIALS AND METHODS

Samples

Fertile hydatid cysts were obtained from sheep and macropod marsupials in the south-west and south-east corners of Australia (Table 1). These two areas are separated by approximately 3500 km. Sheep were slaughtered at abattoirs in a number of localities from each area; we have no information on their origin or previous movements. Macropods were shot in state forests in the same localities.

Protoscoleces were removed from cysts, washed in phosphate-buffered saline (PBS) and stored at −75 °C. If sufficient protoscoleces were obtained, one or more dogs were infected and after 35 days, adult worms were removed using procedures described by Thompson (1977), washed in PBS and stored at −75 °C. The protoscoleces and adults derived from one cyst were regarded as one isolate. The number and size of rostellar hooks were measured for each isolate, as described by Hobbs *et al.* (1990). A total of 65 isolates was obtained from all host species in both geographic areas. The isolates from each type of host (sheep or macropod) in each area (western or eastern Australia) are considered to represent a sample from a natural population (Table 1).

Electrophoresis

Samples were prepared, electrophoresed and stained as described by Lymbery & Thompson (1988). Seventeen enzymes, presumed to be encoded by 23 loci, were examined: adenosine deaminase (encoded by the locus *Ada*); adenylate kinase (*Ak*); esterase (*Est*); glucose-6-phosphate dehydrogenase (*G6pd-1, G6pd-2*); glucose phosphate isomerase (*Gpi*); glutamate dehydrogenase (*Gdh*); glutamate oxaloacetate transaminase (*Got*); hexokinase (*Hk-1, Hk-2*); isocitrate dehydrogenase (*Idh-1, Idh-2*); malate dehydrogenase (*Mdh*); malic enzyme (*Me*); mannose-phosphate isomerase (*Mpi*); nucleoside phosphorylase (*Np-1, Np-2*); leucyl-glycyl-glycine peptidase (*Pep*); phosphoglucomutase (*Pgm*); 6-phosphogluconate dehydrogenase (*6pgd-1, 6pgd-2*); superoxidase dismutase (*Sod-1, Sod-2*).

Enzyme banding patterns were interpreted genetically, as described by Lymbery & Thompson (1988). Loci coding for the enzymes are referred to by italicised, lower-case abbreviations. Multiple loci are designated by number, beginning with the locus responsible for the most anodally migrating isozyme. Alleles are designated by superscripts which represent the mobilities of their respective allozymes relative to that of the most common allozyme among all isolates (which is assigned a mobility value of 100).

Analysis

The genotype of each isolate was determined at as many enzyme loci as possible; some isolates could not be scored for all 23 loci. Genotypic and allelic frequencies were calculated for each population at all loci.

Genetic diversity within populations was described by three standard measures; P, the proportion of polymorphic loci, where a polymorphic locus is one at which the frequency of the most common allele is less than 99%; A, the mean number of alleles at all loci; H, the total gene diversity or expected mean heterozygosity corrected for small sample size, defined as the mean of h over all loci, where $h = (1-\Sigma p_i^2)\ (2N/2N-1)$, p_i is the frequency of the ith allele at a locus and N is the sample size (Nei, 1978). For polymorphic loci, genotypic frequencies expected under Hardy-Weinberg equilibrium were calculated from allelic frequencies using Levene's (1949) correction for small sample size. Deviations of observed from expected genotypic frequencies were analysed by the log likelihood ratio (G) test. To test for association between alleles at different polymorphic loci, observed multi-locus genotypic frequencies were compared with those expected from single-locus frequencies, using the G-test (Allard *et al.* 1972). For all G-tests, genotypic classes with expected

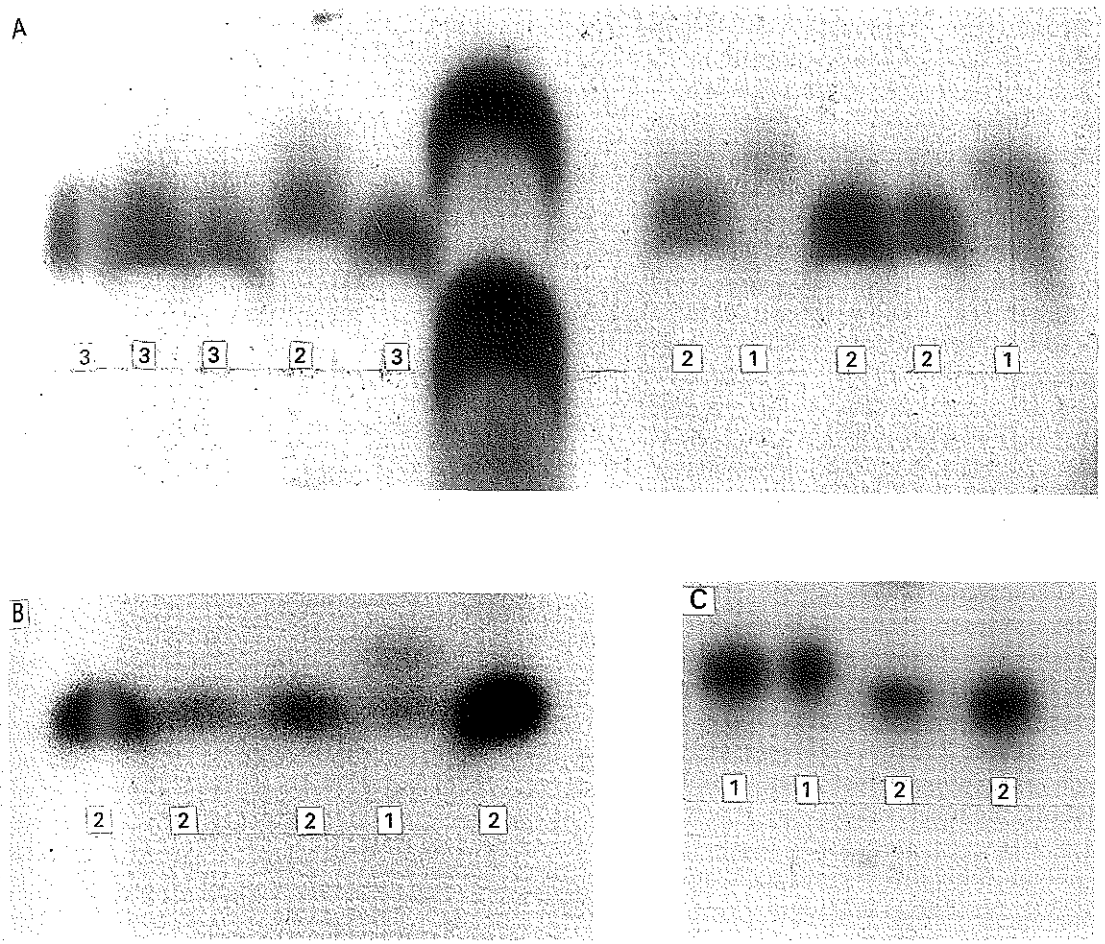

Fig. 1. Electrophoretic patterns in isolates of *Echinococcus granulosus* from macropods in Western Australia. (A) Variation at the *Est* locus. Presumed genotypes; Est^{112}/Est^{112} (1), Est^{100}/Est^{100} (2), Est^{95}/Est^{95} (3). Lane 6 shows enzymes of host origin. (B) Variation at the *Mpi* locus. Presumed genotypes; Mpi^{107}/Mpi^{100} (1), Mpi^{100}/Mpi^{100} (2). (C) Variation at the *Pep* locus. Presumed genotypes; Pep^{100}/Pep^{100} (1), Pep^{92}/Pep^{92} (2).

frequencies less than 5 were pooled and Yates' correction for continuity was applied where appropriate.

Genetic differentiation between populations was described by Wright's F_{ST} values (Wright, 1951, 1965), calculated by the method of Weir & Cockerham (1984), which explicitly corrects for a small number of populations and for small or unequal sample sizes. F_{ST} provides a measure of the correlation between genes of different individuals in the same population, relative to some more inclusive grouping. Cockerham (1973) demonstrated that F_{ST} is also equivalent to a variance component, describing the proportion of total genetic variance among all individuals due to genetic differences between populations. F_{ST} varies between 0 and 1, with 0 indicating no differentiation and 1 complete differentiation between populations. F_{ST} values were calculated for each polymorphic locus; small negative values, which were occasionally obtained because of sample size corrections, were set to 0. G-tests were used to determine the significance of differences in allelic frequencies between populations at each locus. F_{ST} values were jackknifed over loci to estimate mean and variance (Weir & Cockerham, 1984). Jackknifing is a resampling procedure which allows estimation of a parameter whose distribution is unknown (Efron, 1982). The sequential samples should be independent replicates which, in our case, implies that there is no association between alleles at different polymorphic loci. To determine whether mean values were significantly greater than 0, 95% confidence limits were calculated from standard errors.

To provide a measure of the genetic relationship between populations, Nei's normalized coefficient of genetic identity (I), corrected for small sample sizes (Nei, 1978), was calculated for all pairs of populations from allelic frequencies at all monomorphic and polymorphic loci.

RESULTS

Genetic diversity within populations

Among all isolates, variation was detected at 6 of the 23 loci assayed. In the absence of controlled breeding

Table 2. Proportion of polymorphic loci (P), mean number of alleles per locus (A) and total gene diversity (H) in four populations of *Echinococcus granulosus*

Population*	P	A	H
EAS	0·22	1·3	0·04
EAM	0·17	1·2	0·05
WAS	0·09	1·1	0·02
WAM	0·13	1·2	0·04

* See Table 1 for code.

experiments, we cannot rule out the possibility of post-translational modification of enzymes, but the consistency of the mobility variants, and their correspondence with previously described isozymes (Lymbery & Thompson, 1988, 1990) strongly suggests that they represent the products of different alleles. Three alleles were found for *Est* and 2 alleles for *G6pd-2, Idh-1, Mpi, Np-2* and *Pep*. Fig. 1 shows examples of presumed allelic variation at the *Est*, *Mpi* and *Pep* loci. Table 2 shows estimates of P, A and H for each population which was sampled. Over all populations the mean values were $\bar{P} = 0{\cdot}15$, $\bar{A} = 1{\cdot}2$ and $\bar{H} = 0{\cdot}04$.

The 3 most polymorphic loci over all populations were *Est* ($\bar{h} = 0{\cdot}30$), *Np-2* ($\bar{h} = 0{\cdot}22$) and *Pep* ($\bar{h} = 0{\cdot}11$). Despite the extensive genetic diversity at these loci, only 1 heterozygote was found among all isolates examined. This observed deficiency of heterozygotes was significant in all populations where sample sizes and diversity were great enough to test the deviation of genotypic frequencies from Hardy-Weinberg expectations (for EAS: $G = 12{\cdot}63$, $P < 0{\cdot}001$ at *Est*; $G = 12{\cdot}04$, $P < 0{\cdot}001$ at *Pep*; for EAM: $G = 4{\cdot}56$, $P < 0{\cdot}05$ at *Est*; for WAM: $G = 10{\cdot}70$, $P < 0{\cdot}01$ at *Est*; $G = 11.12$, $P < 0{\cdot}001$ at *Np-2*).

There was no evidence of associations between alleles at the 3 major polymorphic loci. Among all isolates, 6 out of 16 possible 3-locus genotypes were observed. Among isolates from the EAS population (where samples sizes were large enough for statistical analysis), 3-locus genotypic frequencies were not significantly different from those computed from the products of single-locus frequencies ($G = 0{\cdot}41$, $P > 0{\cdot}05$).

Genetic differentiation between populations

Table 3 shows allelic frequencies at the 3 major polymorphic loci in all populations. There was significant heterogeneity in allelic frequencies between populations at 1 locus (Table 4). Over all loci, however, only 5·2% of the variance in allelic frequencies was due to differences between populations (mean $F_{ST} = 0{\cdot}052$, Table 4), which is not significantly different from 0. Effectively all the genetic variation found in this study was between isolates from the same geographic area and host type.

Table 3. Allelic frequencies at three polymorphic loci in four populations of *Echinococcus granulosus*

		Population*			
Locus	Allele	EAS	EAM	WAS	WAM
Est	112	0·06	—	—	0·12
	100	0·83	0·75	0·92	0·82
	95	0·11	0·25	0·08	0·06
Np-2	100	1·00	0·86	0·90	0·69
	88	—	0·14	0·10	0·31
Pep	100	0·86	1·00	1·00	0·90
	92	0·14	—	—	0·10

* See Table 1 for code.

Table 4. F_{ST} valves for three polymorphic loci among four populations of *Echinococcus granulosus*. (Significant heterogeneity in allelic frequencies at *Np-2* indicated by ** ($G = 15{\cdot}12$, $P < 0{\cdot}01$.)

Locus	F_{ST}
Est	0·004
Np-2	0·140**
Pep	0·017
Mean ± standard error†	0·052 ± 0·014
95% confidence interval	−0·007 < 0·052 < 0·111

† Estimated by jackknifing over loci.

Table 5. Coefficients of genetic identity (Nei's I) between populations* of *Echinococcus granulosus*

	EAS	EAM	WAS
EAM	0·998		
WAS	0·999	0·999	
WAM	0·996	0·998	0·998

* See Table 1 for code.

Coefficients of genetic identity, calculated over all loci, indicated a very high degree of genetic similarity ($\bar{I} = 0{\cdot}998$) between all populations (Table 5).

Genetic differentiation between strains

On the basis of total number of rostellar hooks and length of the large hooks, measured on either protoscoleces or adults (Hobbs *et al.* 1990), 33 isolates (29 from sheep and 4 from macropods) conformed to the mainland domestic strain of *E. granulosus*, while 11 isolates (all from macropods) conformed to the mainland sylvatic strain, as described by Kumaratilake & Thompson (1984*a*). Ten

Table 6. F_{ST} values for three polymorphic loci between morphologically defined strains of *Echinococcus granulosus* (Significant difference in allelic frequencies at *Est* indicated by * (G = 5·56, $P < 0·05$.)

Locus	F_{ST}
Est	0·060*
Np-2	0·167
Pep	0·000
Mean ± standard error†	0·061 ± 0·011
95% confidence interval	0·014 < 0·061 < 0·108

† Estimated by jackknifing over loci.

isolates could not be classed as belonging to either strain on the basis of rostellar hook morphology, while 11 isolates were not measured. There was a significant difference in allelic frequencies at 1 locus between isolates typed as domestic or sylvatic strain (Table 6). Over all loci, there was significant heterogeneity between strains, although it accounted for only 6·1% of the total variance in allelic frequencies (mean F_{ST} = 0·061, Table 6). This leaves almost 94% of genetic variation to be explained by differences between isolates within each strain. Nei's coefficient of genetic identity between strains, calculated over all loci, was 0·996.

DISCUSSION

Lymbery & Thompson (1988) reported polymorphism at six enzyme loci among isolates of *E. granulosus* from sheep on the mainland of Australia and in Tasmania. In the present study, we have found polymorphism at four of these loci, and another two (*Np-2* and *Pep*) in isolates from both sheep and macropods on the mainland. Although levels of genetic diversity within populations are less than those reported for most other species of parasitic helminths (Nadler, 1987, 1990), our estimates are probably conservative (Lymbery & Thompson, 1988).

Isozyme and DNA analyses by Le Riche & Sewell (1978), McManus & Smyth (1979), Macpherson & McManus (1982), McManus & Simpson (1985) and McManus & Rishi (1989) have found genetic variation between, but not within, strains of *E. granulosus* in Europe and Africa. This has led to a view of these strains as being completely homozygous and monomorphic, a view thought to be in keeping with Smyth's model of obligate self-fertilization in the species (McManus & Smyth, 1979; Macpherson & McManus, 1982).

The finding of genetic variation within all the previously described Australian strains does not necessarily provide evidence against Smyth's model. Lymbery & Thompson (1988) pointed out that whether *E. granulosus* is normally self-fertilizing or cross-fertilizing may make little difference to the extent of genetic variation within strains; the breeding system can be inferred not from the existence of genetic variation, but from its pattern. In the present study, we found significant deficiencies of heterozygotes in all populations, as would be expected if *E. granulosus* is normally self-fertilizing (Thompson & Lymbery, 1988). On the other hand, these data may be at least partially explained by a Wahlund effect (Lymbery & Thompson, 1988) and the absence of associations between alleles at different loci suggests that cross-fertilization, which recombines alleles and breaks up such associations, may also occur. Sample sizes in the present study were too small to provide reliable estimates of the outcrossing rate in *E. granulosus*. We are presently measuring multilocus variation in a much larger number of isolates, and these data will allow us to more accurately assess the breeding system.

The existence of two strains of *E. granulosus* on the mainland of Australia was first proposed by Kumaratilake & Thompson (1982*b*). Thompson & Kumaratilake (1985) suggested that the strains were introduced separately into Australia, with the domestic strain arriving in sheep brought by European settlers and the sylvatic strain arriving many thousands of years earlier in dingoes brought by Aborigines. The independent origin of the strains would mean that they were adapted to different life-cycles, ensuring partial ecological and geographical separation in Australia. This scenario predicts that most genetic variation in *E. granulosus* on the mainland of Australia should be found between isolates from domestic and sylvatic life-cycles.

Our results do not confirm this prediction. While a substantial amount of genetic variation was uncovered, virtually all of it occurred within populations from the same host type (domestic or sylvatic) and geographic area. The mean genetic identity between natural populations was 0·998, which is higher than values reported for subspecific populations of most other organisms which have been studied (Ayala, 1975; Thorpe, 1982, 1983).

Kumaratilake & Thompson (1984*a*, *b*) argued that, although the two strains of *E. granulosus* on the mainland of Australia were largely distinct, both ecologically and genetically, macropod marsupials sometimes harboured the domestic strain. If this occurred commonly in the areas we sampled, it could explain the lack of differentiation between populations in different hosts, even if different strains were present. There was significant genetic variation between isolates characterized as domestic or sylvatic, using the rostellar morphological criteria of Kumaratilake & Thompson (1984*a*), but even so it accounted for only about 6% of the total; 94% of genetic variation occurred within the putative

strains. Furthermore, the differences which were present cannot be confidently interpreted as resulting from a restriction in gene flow between isolates in domestic and sylvatic cycles, because host origin is confounded with geographic origin in such an analysis. Genetic identity between the strains (I = 0·996) was of the same order as that between natural populations.

The simplest interpretation of these data is that, while there may be substantial genetic diversity among isolates of *E. granulosus* on the mainland of Australia, there is little evidence of genetic differentiation between populations in domestic and sylvatic life-cycles. This clearly conflicts with the conclusions of Kumaratilake & Thompson (1982*b*; 1983, 1984*a*, *b*) and Thompson & Kumaratilake (1985), who argued that the discontinuous nature of differences between isolates from sylvatic and domestic hosts in rostellar and strobilar morphology, in the banding patterns of soluble proteins and in the development rate of secondary cysts and adult worms, implied an underlying genetic separation.

There are two ways in which these conflicting interpretations could be reconciled. Firstly, some or all of the sylvatic isolates studied by Kumaratilake & Thompson may have come from an isolated relict population, genetically distinct from the populations we sampled. Our sampling sites did not include all the areas from which sylvatic isolates were received in previous studies and we have no evidence that the populations we sampled from sylvatic hosts had been maintained in predominantly sylvatic life-cycles. Secondly, the characters studied by Kumaratilake and Thompson may have been influenced by the environment of the intermediate host. This was suggested by our study of rostellar morphology (Hobbs *et al.* 1990), and may also be true for strobilar morphology, development rate and even protein banding patterns, as there is no information on the relative magnitude of genetic and environmental components of variance in these phenotypic traits.

At this stage, we cannot conclude that the domestic and sylvatic strains of *E. granulosus* proposed by Kumaratilake & Thompson (1984*a*, *b*) and Thompson & Kumaratilake (1985) do not exist. It seems unlikely that the differences they described reflect a fundamental genetic disjunction between all populations in different life-cycles on the mainland of Australia. However, it is possible that a genetically distinct sylvatic strain exists in isolated sylvatic life-cycles. Future studies should examine isolates from sylvatic hosts in the areas sampled by Kumaratilake & Thompson (1983, 1984*a*, *b*).

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REFERENCES

ALLARD, R. W., BABBEL, G. R., CLEGG, M. T., KAHLER, A. L. (1972). Evidence for coadaptation in *Avena barbata*. *Proceedings of the National Academy of Sciences, USA* **69**, 3043–8.

AYALA, F. J. (1975). Genetic differentiation during the speciation process. *Evolutionary Biology* **8**, 1–78.

COCKERHAM, C. C. (1973). Analyses of gene frequencies. *Genetics* **74**, 679–700.

EFRON, B. (1982). *The Jackknife, the Bootstrap and Other Resampling Plans*. Philadelphia: Society for Industrial and Applied Mathematics.

HOBBS, R. P., LYMBERY, A. J. & THOMPSON, R. C. A. (1990). Rostellar hook morphology of *Echinococcus granulosus* (Batsch, 1786) from natural and experimental Australian hosts. *Parasitology* **101**, 273–281.

KUMARATILAKE, L. M. & THOMPSON, R. C. A. (1982*a*). A review of the taxonomy and speciation of the genus *Echinococcus* Rudolphi 1801. *Zeitschrift für Parasitenkunde* **68**, 121–46.

KUMARATILAKE, L. M. & THOMPSON, R. C. A. (1982*b*). Hydatidosis/echinococcosis in Australia. *Helminthological Abstracts A, Animal and Human Helminthology* **51**, 233–52.

KUMARATILAKE, L. M. & THOMPSON, R. C. A. (1983). A comparison of *Echinococcus granulosus* from different geographical areas of Australia using secondary cyst development in mice. *International Journal for Parasitology* **13**, 509–15.

KUMARATILAKE, L. M. & THOMPSON, R. C. A. (1984*a*). Morphological characterisation of Australian strains of *Echinococcus granulosus*. *International Journal for Parasitology* **14**, 467–77.

KUMARATILAKE, L. M. & THOMPSON, R. C. A. (1984*b*). Biochemical characterisation of Australian strains of *Echinococcus granulosus* by isoelectric focusing of soluble proteins. *International Journal for Parasitology* **14**, 581–6.

LE RICHE, P. D. & SEWELL, M. M. H. (1978). Identification of *Echinococcus granulosus* strains by enzyme electrophoresis. *Research in Veterinary Science* **25**, 247–8.

LEVENE, H. (1949). On a matching problem arising in genetics. *Annals of Mathematical Statistics* **20**, 91–4.

LYMBERY, A. J. & THOMPSON, R. C. A. (1988). Electrophoretic analysis of genetic variation in *Echinococcus granulosus* from domestic hosts in Australia. *International Journal for Parasitology* **18**, 803–11.

LYMBERY, A. J. & THOMPSON, R. C. A. (1990). Genetic differences between cysts of *Echinococcus granulosus* from the same host. *International Journal for Parasitology* **19**, 961–4.

MACPHERSON, C. N. L. & MCMANUS, D. P. (1982). A comparative study of *Echinococcus granulosus* from human and animal hosts in Kenya using isoelectric focusing and isoenzyme analysis. *International Journal for Parasitology* **12**, 515–21.

McMANUS, D. P. & SMYTH, J. D. (1979). Isoelectric focusing of some enzymes from *Echinococcus granulosus* (horse and sheep strains) and *E. multilocularis*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **73**, 259–65.

McMANUS, D. P. & SIMPSON, A. J. G. (1985). Identification of the *Echinococcus* (hydatid disease) organisms using cloned DNA markers. *Molecular and Biochemical Parasitology* **17**, 171–8.

McMANUS, D. P. & SMYTH, J. D. (1986). Hydatidosis: changing concepts in epidemiology and speciation. *Parasitology Today* **2**, 163–8.

McMANUS, D. P. & RISHI, A. K. (1989). Genetic heterogeneity within *Echinococcus granulosus* isolates from different hosts and geographical areas characterized with DNA probes. *Parasitology* **99**, 17–29.

NADLER, S. A. (1987). Genetic variability in endoparasitic helminths. *Parasitology Today* **3**, 154–5.

NADLER, S. A. (1990). Molecular approaches to studying helminth population genetics and phylogeny. *International Journal for Parasitology* **20**, 11–29.

NEI, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–90.

RAUSCH, R. L. (1967). A consideration of infraspecific categories in the genus *Echinococcus* Rudolphi, 1801 (Cestoda: Taeniidae). *Journal of Parasitology* **53**, 484–91.

RAUSCH, R. L. (1985). Parasitology: retrospect and prospect. *Journal of Parasitology* **71**, 139–51.

SMYTH, J. D. & SMYTH, M. M. (1964). Natural and experimental hosts of *Echinococcus granulosus* and *E. multilocularis*, with comments on the genetics of speciation in the genus *Echinococcus*. *Parasitology* **54**, 493–514.

THOMPSON, R. C. A. (1977). Growth, segmentation and maturation of the British horse and sheep strains of *Echinococcus granulosus* in dogs. *International Journal for Parasitology* **7**, 281–5.

THOMPSON, R. C. A. & KUMARATILAKE, L. M. (1985). Comparative development of Australian strains of *Echinococcus granulosus* in dingoes (*Canis familiaris dingo*) and domestic dogs (*C. f. familiaris*), with further evidence for the origin of the Australian sylvatic strain. *International Journal for Parasitology* **15**, 535–42.

THOMPSON, R. C. A. & LYMBERY, A. J. (1988). The nature, extent and significance of variation within the genus *Echinococcus*. *Advances in Parasitology* **27**, 209–58.

THORPE, J. P. (1982). The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. *Annual Review of Ecology and Systematics* **13**, 139–68.

THORPE, J. P. (1983). Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. In *Protein Polymorphism: Adaptive and Taxonomic Significance* (ed. Oxford, G. S. & Rollinson, D.), pp. 131–152. London: Academic Press.

WEIR, B. S. & COCKERHAM, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–70.

WRIGHT, S. (1951). The genetical structure of populations. *Annals of Eugenics* **15**, 323–54.

WRIGHT, S. (1965). The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* **19**, 395–420.