The genetic structure of *Escherichia coli* populations in feral house mice

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***Escherichia coli* was isolated from feral house mice (*Mus domesticus*) during the course of a mouse plague in the state of Victoria, Australia. Two farms were sampled over a period of 7 months and a total of 447 isolates were collected. The isolates were characterized using the techniques of randomly amplified polymorphic DNA and multi-locus enzyme electrophoresis. The mean genetic diversity of this *E. coli* population ($H = 0.24$) was found to be substantially lower than the diversity of an *E. coli* population reported elsewhere for a single human host. Analysis of the allozyme data revealed that there were significant differences in the relative abundance of genotypes between the two localities sampled and among sample dates. Overall, however, spatial and temporal effects accounted for less than 5% of the genotypic diversity. Allele frequencies and the relative abundance of the more common genotypes did not differ between male and female hosts. The number of genotypes and genotype diversity increased as the age of the host increased, suggesting that the mice are continuing to acquire new *E. coli* clones throughout their life. The frequency of some alleles changed with respect to host age, which indicates that clone acquisition may not be a random process. It is argued that the low level of genetic diversity observed in this population of *E. coli* reflects the boom and bust nature of mouse population density in this region of Australia.**

Keywords: *Escherichia coli*, allozymes, genotypic diversity, population genetics, mouse

INTRODUCTION

Studies concerning *Escherichia coli* have formed the empirical basis of our understanding of the genetic structure of bacterial populations (Milkman, 1973; Selander & Levin, 1980; Whittam *et al.*, 1983a, b; Miller & Hartl, 1986; Maynard Smith *et al.*, 1993; Guttman & Dykuizen, 1994). Many of these studies have focused on clinical isolates responsible for a variety of diseases (Caugant *et al.*, 1983; Selander *et al.*, 1986b; Whittam *et al.*, 1993). Some have examined issues related to clonal diversity and turnover in a single host (Caugant *et al.*, 1981) or the degree to which strains are shared between hosts (Caugant *et al.*, 1984). Virtually all of these studies have been restricted to *E. coli* isolated from humans. Notable exceptions to the observed bias towards human isolates do exist but they are few in number (Routman *et al.*, 1985; Whittam, 1989). An additional major bias present in many previous studies has been their basis on

ad hoc collections of isolates of diverse geographic origin. Few studies have examined the structure of *E. coli* populations from a single host species or geographic locality.

The purpose of this study was to examine the genetic structure of *E. coli* from feral house mouse (*Mus domesticus*) populations and to investigate the role that host, spatial and temporal factors play in determining the structure of these populations. Collections were made in a region of Australia where house mouse population numbers regularly attain plague status, an event which occurred during this study. Over 440 strains of *E. coli* were isolated from two localities over a 7 month period and characterized using the techniques of randomly amplified polymorphic DNA (RAPD) and multi-locus enzyme electrophoresis (MLEE).

METHODS

Study site and host collection. Mice were collected from two wheat-growing properties (Stone and Symes) 15 km apart near the town of Walpeup in the Mallee district of north western

Abbreviations: ETs, electrophoretic types; MLEE, multi-locus enzyme electrophoresis; RAPD, randomly amplified polymorphic DNA.

Table 1. Details of mouse samples

Sample criteria		Number of mice in sample	
		Stone	Symes
Date	Dec 1993	26	23
	Jan 1994	42	37
	Feb 1994	33	37
	Mar 1994	32	30
	Apr 1994	49	49
	Jun 1994	44	45
Host sex	Male	129	139
	Female	97	82
Host age	< 5 weeks	34	54
	5–8 weeks	52	79
	> 8 weeks	140	88

Victoria, Australia. There are no discernible differences between the two study sites in terms of topography, vegetation, land use or the proximity to human habitation. Trapping transects were established in a single field on each property. At intervals throughout the summer and autumn of 1993/94, 100 live traps were placed along each transect in the evening and checked the following morning. Efforts were made to trap 50 mice per site on every sampling occasion. The required number of animals were usually captured in a single night.

A total of 447 mice were sampled. The numbers of hosts partitioned by sample site, date, sex and age are presented in Table 1. Males represent 60% of the mice collected, a bias resulting from the fact that males are more active and move over a greater area than females such that males are more susceptible to capture. The age structure of the host population varied between sites with adults accounting for 62% of the captures at the Stone site and 40% at Symes. The population at Symes started breeding approximately 1 month later than at Stone and this explains the greater proportion of young mice captured at Symes. Mouse population density peaked in April/May at both localities.

Isolation and characterization of bacteria. The hosts were killed and eviscerated at the field laboratory. The large intestine was opened aseptically and a sample of the upper colon contents was taken using a sterile swab. The swabs were placed in Aimes transport agar (Power & McCuen, 1988) and stored at 5 °C until processed.

A single presumptive *E. coli* clone was isolated from every animal by streaking the swab onto a MacConkey agar plate (Power & McCuen, 1988). Every isolate was subsequently purified by subculture onto another MacConkey agar plate followed by an LB agar plate (Power & McCuen, 1988). All isolates were tested for growth on minimal lactose and minimal citrate agar plates (Power & McCuen, 1988) and the Lac⁺ Cit⁻ isolates presumed to be *E. coli*. The isolates were then stored in glycerol at -80 °C. Subsequently all isolates were subjected to further biochemical tests and found to exhibit characteristics typical of *E. coli* (Ewing, 1986; Power & McCuen, 1988). All incubations were carried out at 37 °C.

Genotypic characterization of strains. Two 10-mer primers were used in the RAPD analysis: B01, 5' GTTTCGCTCC; and A19, 5' CAAACGTCGG (Operon Technologies). Tem-

plate DNA was prepared by pelleting 1 ml of an overnight LB culture and resuspending it in 50 µl sterile ultra-pure water. Cells were lysed by alternate 1 min cycles in boiling water and liquid nitrogen. The cellular debris was pelleted and 1 µl of the supernatant used per reaction. Reaction volumes were 10 µl and the reaction mix consisted of: 67 mM Tris/HCl, pH 8.8, 16.6 mM (NH₄)₂SO₄, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.45% Triton X-100, 0.2 mg gelatin ml⁻¹, 3.1 ng primer µl⁻¹. PCR amplification conditions were: 5 cycles of 92 °C for 10 s, 40 °C for 120 s and 72 °C for 90 s, then 35 cycles of 92 °C for 5 s, 45 °C for 25 s and 72 °C for 90 s and finally 1 cycle of 92 °C for 10 s, 45 °C for 20 s and 72 °C for 300 s. The resulting PCR product was visualized on ethidium-bromide-stained 6% polyacrylamide gels. This procedure was repeated at least twice for each strain to confirm its genotype.

MLEE assays were run using Titan III cellulose acetate gels (Hebert & Beaton, 1993). Protein extracts were prepared by growing the strains in 20 ml LB at 37 °C overnight. The cells were pelleted and washed twice in 0.15 M potassium phosphate buffer, pH 7.0, and then resuspended in 50 µl lysis buffer (l⁻¹: 100 mg NADP, 0.5 ml 2-mercaptoethanol). The cell suspension was sonicated in three 5 s bursts while being kept chilled on ice. The sonicated suspension was centrifuged at 4 °C and 13000 r.p.m. for 20 min and the supernatant was stored at -80 °C. The following enzyme systems were used: ADH (alcohol dehydrogenase EC 1.1.1.1), AK (adenylate kinase EC 2.7.4.3), G6PD (glucose-6-phosphate dehydrogenase EC 1.1.1.49), MDH (malate dehydrogenase EC 1.1.1.37), MPI (mannose phosphate isomerase EC 5.3.1.8), MR (menadione reductase EC 1.6.99.2), 6PGD (6-phosphogluconate dehydrogenase EC 1.1.1.44), GPI (phosphoglucose isomerase EC 5.3.1.9), PGM (phosphoglucomutase EC 5.4.2.2), SDH (shikimate dehydrogenase EC 1.1.1.25) and SOD (superoxide dismutase EC 1.15.1.1). MPI, GPI and PGM were run in 0.025 M Tris/glycine buffer, pH 8.5; all others were run in 0.1 M Tris/maleate buffer, pH 7.8 (Hebert & Beaton 1989). All strains were run at least twice to confirm their genotype.

Statistical analyses. The genetic distances between the strains were calculated as $D = 1 - [2m_{xy}/(m_x + m_y)]$ where m_{xy} is the number of RAPD bands (or alleles) shared by isolates x and y , while m_x and m_y are the total number of bands (or alleles) present in isolates x and y , respectively (Nei & Li, 1979). RAPD and MLEE distance matrices were compared using the Mantel test (Rohlf, 1993). Phenograms were constructed from the distance matrices using the unweighted pair group method (UPGMA) (Rohlf, 1993). The reliability of the phenograms was determined by bootstrapping (Rohlf, 1993) as follows. Loci (MLEE) or bands (RAPD) were randomly selected, with replacement, to produce 50 replicated data sets of the same size as the original data sets. New distances matrices were then calculated and subjected to UPGMA analysis. Comparisons of the bootstrapped phenograms or RAPD and MLEE phenograms were made using the Strict Consensus method (Rohlf, 1993).

The total number of genotypes in the population was estimated by drawing 50 random samples of isolates of varying size (S) with replacement and then determining the number of MLEE genotypes present in each random sample (Selander *et al.*, 1987). The resulting data were then described by the following monotonic function: $ETs = \alpha[1 - \exp(-\beta S)]^{(1/\delta)}$, where ETs denote the number of genotypes in a sample of size S . The parameters β and δ determine the shape of the curve and α the asymptote, or the estimated total number of genotypes in the population.

Genotypic diversity was estimated as $G = (1 - \sum g_i^2)n/(n-1)$ where g is the frequency of genotype i and n is the total number of isolates. The proportion of genotypic diversity attributable to a particular effect (e.g. locality) is $(G_T - G_s)/G_T$, where G_s is the arithmetic mean of G calculated separately for each locality and G_T is the diversity of all isolates regardless of locality (Nei, 1978; Selander *et al.*, 1986a).

The frequency of the i th allele at the j th locus is p_{ij} . The probability that two individuals differ at the j th locus is $h_j = (1 - \sum p_{ij}^2)n/(n-1)$. The mean genetic diversity, H , is the arithmetic mean of h_j for m loci (Nei, 1978; Selander *et al.*, 1986a).

Estimates of multi-locus linkage disequilibrium were based on the index developed by Brown *et al.* (1980). K is the total number of loci at which two individuals differ, there are $n(n-1)/2$ such pairs in a sample of n individuals. The mean difference between two individuals is $K = \sum h_j$. In the absence of linkage disequilibrium the expected variance of K is $V_e = \sum h_j(1-h_j)$. The observed variance of K is V_o and the index of linkage disequilibrium is V_o/V_e . To test if this ratio is significantly greater than one, a randomization test was employed. Samples of the same size as the original data set were generated by randomly sampling alleles, without replacement, according to their respective frequencies at each locus (Souza *et al.*, 1993). For each random sample V_o and V_e are calculated and the probability of observing a V_o/V_e ratio as extreme as that for the original data is determined.

Analysis of deviance was used to compare genotype or allele frequencies with respect to locality, sample date, host age or sex. There is a bias inherent in the data as a result of the different age structure of the host populations at the two farms – a smaller proportion of adults at Symes. As a consequence, comparisons were adjusted for this bias where appropriate. Uncommon genotypes or rare alleles were not included in the analyses.

RESULTS

RAPD versus MLEE results and clonal structure

The two RAPD primers produced a total of 47 bands with a mean of six bands per strain. Among the 447 isolates there were 50 different RAPD genotypes. The 13 allozyme loci revealed a total of 43 alleles giving rise to 60 unique MLEE types. Pooling the RAPD and MLEE data resulted in a total of 88 genotypes.

Strains with identical RAPD types had different MLEE profiles while the converse was also true. Of the 50 RAPD types, 64% represented a single MLEE type, while one of the RAPD types encompassed as many as 10 MLEE types (Fig. 1). The corresponding MLEE comparison revealed that 80% of the MLEE types represented a single RAPD type. There were two MLEE types each of which was composed of six different RAPD types (Fig. 1).

The results of a UPGMA cluster analysis for the 88 genotypes based on the RAPD data and the MLEE results are presented in Fig. 1. Bootstrap analysis revealed that relatively little confidence can be placed on most of the nodes in either of the phenograms. The only consensus between the phenograms are the two clusters

of strains indicated in Fig. 1. Cluster B consists of two genotypes, each with a unique RAPD and MLEE profile. Cluster A consists of 12 genotypes, consisting of five unique RAPD types and eight different MLEE types. The bootstrap analysis shows that these groups of strains consistently cluster in both UPGMA analyses (Fig. 1).

The degree of genetic similarity among the 88 genotypes implied by the RAPD and MLEE data are comparable. There was modest but significant correlation between the distance matrices derived from the two sets of data ($R = 0.48$, $P < 0.001$). Further support for the conclusion that both data sets imply similar genetic distances between the strains is given by the following analysis. On average, the 88 genotypes differed at four MLEE loci. There were significantly fewer differences (2.5) between different MLEE types with the same RAPD profile (median test, $P < 0.001$).

An analysis of multi-locus linkage disequilibrium based on the MLEE data and considering the 88 genotypes revealed a significant degree of linkage disequilibrium ($V_o/V_e = 1.76$, $P < 0.001$). When the analysis was restricted to the 12 genotypes forming cluster A no linkage disequilibrium could be detected ($V_o/V_e = 0.98$, $P > 0.45$). Similarly, when the analysis included all of the genotypes not present in clusters A or B ($N = 74$), there was little evidence for linkage disequilibrium ($V_o/V_e = 1.21$, $P > 0.03$).

Genotypic diversity: temporal, spatial and host effects

The following analyses have been based on only the allozyme data for three reasons. Firstly, most previous studies concerning the genetic structure of bacterial populations are based on MLEE data. Secondly, basing the analysis on the 88 genotypes resulting from pooling the RAPD and MLEE data results in a significant reduction in the number of times a particular genotype occurs in the sample. Finally, essentially the same outcomes are observed when the analyses are based on the RAPD data or the pooled RAPD and MLEE data.

Of the 60 MLEE types (ETs), 58% were isolated only once while the three most common genotypes represented 48% of the isolates. Repeated random sampling of the isolates produced an estimated number of ETs of $\alpha = 55 \pm 2.3$ (Fig. 2). As 60 ETs were identified, this suggests that all of the common ETs occurring in house mice at the two localities were recovered.

There were 46 ETs recovered from the Stone site and 34 from the Symes locality. Of the 25 ETs recovered more than once, 20% were present at only one of the sites: one absent from Stone and four absent from Symes. Considering only the 10 most abundant ETs, representing 75% of the 447 isolates, there was a significant difference in their frequency of occurrence between sites ($\chi^2 = 55.9$, $P < 0.001$).

Although there were significant differences in the relative abundance of the 10 most common ETs across sample

Fig. 1. Phenograms depicting the genetic similarities among *E. coli* strains isolated from feral house mice. In the phenogram based on RAPD data, strains connected by a vertical line represent strains with identical RAPD profiles but different MLEE profiles. The converse is true for the phenogram based on MLEE data. The numbers represent the isolate used as an example of the genotype (combined RAPD and MLEE results) and the bracketed figures present the number of times each of the genotypes were recovered. The vertical lines labelled A or B encompass those strains which clustered together in both the RAPD and MLEE phenograms. The phenograms were constructed using the UPGMA method. The bootstrap values for nodes are presented for only those clusters of strains which occurred more than 80% of the time.

Fig. 2. Estimating the number of *E. coli* MLEE genotypes in the house mouse population. The symbols represent the number of genotypes that were present in random samples of size *S* and the solid line depicts the fit of the empirical model. See Results for further details.

Table 2. Changes with host age in the genotypic diversity of *E. coli* isolated from house mice

Host age	Richness*	Evenness†
Juvenile	25	10.8
Sub-adult	30	11.7
Adult	44	13.5

* The number of genotypes (ETs) recovered from each host group.

† Simpson's Index = $\Sigma(n_i(n_i - 1)) / (N(N - 1))$ where n_i is the number of isolates of the *i*th ET and *N* is the total number of isolates.

dates ($\chi^2 = 92.49$, $P < 0.001$) no consistent trends could be observed. Some genotypes were recovered on every sampling occasion, some intermittently, and others were only observed in the early samples or the later samples.

No differences between the sexes could be detected in the relative frequency of the 10 most common ETs ($\chi^2 = 11.77$, $P > 0.22$). There were, however, significant differences in the relative frequencies of the 10 most common clones among the different ages of host ($\chi^2 = 35.6$, $P < 0.008$). Much of the age effect appears to be due to an increase in genotypic diversity with increasing host age. The number of genotypes recovered from each age class (richness) and the relative frequencies of the genotypes (evenness) increased as hosts aged (Table 2).

The total genotypic diversity, G_T – the probability of two randomly selected clones being different – was 0.91. Although there were significant differences in the relative abundances of the 60 ETs due to spatial, temporal and host effects, individually these factors did not account for any more than 5% of the total genotypic diversity.

Allelic diversity: temporal, spatial and host effects

There was an average of 3.3 alleles per locus. The allelic diversity at each locus is presented in Table 3. The mean allelic diversity across all loci was $H = 0.24$ (isolates)

Table 3. Results of the allozyme analysis of 447 *E. coli* strains isolated from feral house mice

Locus	No. of alleles	h_i^* isolates	$h_i^\dagger$ ETs
ADH	4	0.434	0.517
AK	3	0.428	0.380
G6PD	2	0.013	0.095
MDH	2	0.009	0.064
MPI	4	0.547	0.661
MR1	3	0.272	0.445
MR2	3	0.077	0.209
MR4	2	0.009	0.064
6PGD	4	0.336	0.576
GPI	6	0.526	0.632
PGM	2	0.031	0.095
SDH	5	0.336	0.463
SOD	3	0.135	0.127
Mean	3.3	0.24	0.33

* Genetic diversity calculated using all 447 isolates.

† Genetic diversity based on the 60 MLEE genotypes.

and $H = 0.33$ (ETs). Significant differences between localities in the frequencies of one or more alleles were detected for the following loci; ADH ($\chi^2 = 10.3$, $P < 0.006$), AK ($\chi^2 = 17.1$, $P < 0.001$), MPI ($\chi^2 = 22.7$, $P < 0.001$) and 6PGD ($\chi^2 = 10.4$, $P < 0.01$). Of the 43 alleles present, 16% were absent from one of the localities, six absent from Symes and one from Stone. Significant differences in allele frequencies among sample dates were detected for AK ($\chi^2 = 19.2$, $P < 0.04$), MPI ($\chi^2 = 20.5$, $P < 0.03$) and GPI ($\chi^2 = 22.4$, $P < 0.02$). No trends in the frequency of any allele through time could be observed. No differences in allele frequencies as a function of host sex were detected at any locus. Allele frequencies varied with respect to the age of the host for SDH and MPI (Table 4). As the age of the host increased the frequency of allele 4 declined while the frequency of allele 1 increased for MPI ($\chi^2 = 13.1$, $P < 0.02$). Allele 5 for SDH declined as host age increased ($\chi^2 = 10.2$, $P < 0.04$).

DISCUSSION

The genetic diversity of this population of *E. coli* inhabiting house mice is 0.24. By comparison, the diversity of the *E. coli* isolates taken from a single human host over a period of 11 months was found to be 0.47 (Caugant *et al.*, 1981). Typically, genetic diversity estimates of *E. coli* isolated from human faecal flora range from 0.45 to 0.54 (Selander *et al.*, 1987).

It is likely that numerous factors are responsible for the low level of genetic diversity in *E. coli* of house mice at these localities. It is thought that the natural life cycle of *E. coli* consists of transitions between two distinct habitats (Savageau, 1983). The lower intestine of its animal hosts represents its primary habitat while the environment external to the host (soil, water) represents

Table 4. Changes in allele frequencies among *E. coli* isolated from hosts of different ages

Locus	Allele	Allele frequency (%)		
		Juveniles (<i>n</i> = 88)	Sub-adults (<i>n</i> = 128)	Adults (<i>n</i> = 226)
MPI	1	11	15	26
	3	17	18	17
	4	72	67	57
SDH	1	0	2	7
	2	2	5	8
	4	95	82	77
	5	3	11	8

its secondary habitat. Much of the diversity in the clonal composition of human *E. coli* populations is thought to be due to the continuous acquisition and extinction of clones. Clones are acquired through the ingestion of bacteria from the secondary habitat (primarily contaminated food) and are lost as a result of chance extinction and competition among clones within the host (Caugant *et al.*, 1981; Whittam, 1989). In the region where this study was undertaken, human population densities are low and sewage treatment is of a high standard. The mammal community of the region is depauperate, consisting of very few native or domestic species and during plague years mice are certainly the most abundant mammal species in the region. Thus, most inputs to populations in the secondary habitat may be due to mice. Mouse population densities fluctuate dramatically, such that densities at the peak of a plague and densities during the inter-plague low can differ by 2–4 orders of magnitude (Berry & Bronson, 1992). The same environmental conditions that inhibit mouse population growth between plagues (low rainfall and high temperatures) will probably also reduce the survival of the *E. coli* population in the secondary environment. Thus during inter-plague periods, which typically are of 3–4 years duration (Singleton & Redhead, 1990; Berry & Bronson, 1992), the lack of primary habitat (low host density) and poor survival in the secondary habitat may result in high rates of clone extinction, thereby significantly reducing diversity. In addition, when conditions favourable to a plague do occur mouse population numbers quickly increase (Singleton, 1989). At this time there may be intense selection for clones with the ability to rapidly colonize the primary habitat and this may further reduce genetic diversity. Clonal diversity of the *E. coli* population inhabiting the gut increases with host age, suggesting that mice continue to acquire clones throughout their life. That the acquisition of these clones is not a random process is suggested by the observation that allele frequencies for MPI and SDH differ among strains isolated from hosts of different ages (Table 4).

Significant differences in allele frequencies were detected among the six sample dates. However, the differences were only marginally significant ($0.01 < P < 0.05$) and no systematic temporal trend in the frequency of any

allele could be detected. Over the course of the study there were obvious seasonal changes in the environment and significant changes in the density of the mouse populations. However, it is not possible to determine whether these changes caused the temporal shifts in allele frequencies or whether these differences simply reflect sampling artefacts. In the study of an *E. coli* population from a sewage outflow, Pupo & Richardson (1995) found significant fluctuations in the frequency of several alleles with time. Although they could not explain this, they concluded that the fluctuations were not due to sampling artefacts or temporal variation in the temperature or pH of the sewage outflow.

The RAPD and MLEE analyses gave broadly comparable results in describing the genetic similarity of the strains, producing similar UPGMA phenograms and a significant correlation between the distance matrices. Furthermore, strains with identical RAPD types differed at fewer MLEE loci than would be expected by chance. In an analysis of the strains in the *E. coli* reference collection (ECOR; Ochman & Selander, 1984) a similar correspondence between RAPD and MLEE results was also found (Desjardins *et al.*, 1995). Here we observed that 80% of the MLEE types represented a single RAPD type while only 64% of the RAPD types represented a single MLEE type, and this is primarily a consequence of the lower resolving power of the RAPD analysis. The two primers identified fewer (50) unique genotypes than the 13 loci examined in the MLEE analysis (60). RAPD analysis has been shown to be more capable of discriminating isolates than MLEE analysis in some circumstances (Wang *et al.*, 1993). However, the sensitivity of the RAPD method will depend on the choice and number of primers used in the analysis. In this study, the RAPD primers were not selected for their ability to differentiate between isolates with identical MLEE profiles.

Analysis of the 88 genotypes identified on the basis of the combined RAPD and MLEE results revealed a highly significant level of linkage disequilibrium. Little evidence of linkage disequilibrium could be detected when the genotypes were partitioned on the basis of the UPGMA analysis. Gordon *et al.* (1995) discussed the problems associated with estimating linkage dis-

equilibrium in subsets of strains identified on the basis of cluster analysis of MLEE data. In essence, the problem relates to using the same data to first identify strain clusters and then to estimate the amount of linkage disequilibrium among strains within clusters. It is not always possible to determine if the lack of linkage disequilibrium within subgroups of strains reflects recombination or the limitations inherent in the multi-locus linkage disequilibrium index. The ability of the index to detect disequilibrium tends to decline as H declines, and lower values of diversity are an inevitable consequence of selecting subsets of strains identified using cluster analysis. This bias can never be completely eliminated. Although the RAPD data provide 'independent' evidence for the clustering patterns observed using the MLEE data, the inherent statistical problems associated with analysing strain clusters remain.

Caugant *et al.* (1981) described a method for detecting recombination among strains within a population. Their method looked at those pairs of strains which differed at a single allozyme locus together with the plasmid profiles of those strains. If the strain pairs had very different plasmid profiles they considered this as evidence that the strains diverged prior to establishing in the host population. If the pairs had similar or identical profiles and one strain of the pair had a unique allele, they concluded that the difference between the strains arose due to mutation at that locus. However, if the plasmid profiles were similar or identical and the allele was present in other strains in the population, they considered this as evidence for recombination. Analysis of plasmid profiles in this population of *E. coli* (unpublished data) suggests that recombination can only be invoked as an explanation for < 5% of those strain pairs differing at a single locus. The evidence seems to suggest that the apparent absence of linkage disequilibrium within the subgroups of strains may be due to the limitations of the statistic V_o/V_e rather than significant recombination.

There have been numerous studies concerning the genetic structure of *E. coli* populations and tests for multi-locus linkage equilibrium in these populations have consistently revealed significant levels of linkage disequilibrium. The one exception, has been the sewage outflow population of *E. coli* studied by Pupo & Richardson (1995). Although they did observe linkage disequilibrium for some pairwise loci comparisons, when their data are analysed using a multi-locus estimate no linkage disequilibrium can be detected ($V_o/V_e = 0.98$, based on ETs). In a study of *E. coli* isolated from chickens and from the environment in which the birds were living, Whittam (1989) found that only 10% of the more than 100 clones identified were recovered from both habitats. The extent to which clones recovered from sewage outflows represent clones from the primary habitat (humans) or from the secondary habitat (waste traps, wall populations of sewage pipes, etc.) is not known. If sewage outflows are dominated by clones from the secondary habitat it is possible that not only does the clonal composition of primary and secondary

habitats differ but that there are also real differences in the genetic structure of populations from the two habitats.

Genotypes were not distributed randomly among the two localities. The differences between localities may be a consequence of house mouse population dynamics. As the mouse population density peaks in a plague year, movements in search of food and increased levels of intra-specific aggression lead to marked increases in the rate at which mice disperse (Berry & Bronson, 1992). High levels of dispersal could be expected to minimize differences in the clonal composition of *E. coli* populations between localities. However, during inter-plague periods when mouse population size is low the home range of breeding pairs is quite small and little dispersal occurs (Krebs *et al.*, 1994). In addition, the spatial distribution of the mice becomes far more patchy during the inter-plague periods as mice are restricted to small areas of favourable habitat (Singleton, 1989). As previously suggested, clone extinction rates may be quite high between plagues. Low dispersal rates may fail to homogenize the differences arising between localities as a result of clone extinction. Furthermore, these extinction events may not be entirely random. The composition of the *E. coli* population at Symes did not so much represent a 'different' population from the one at Stone as it represented a subset of the Stone population. Overall, fewer genotypes were recovered from Symes and two of the more common genotypes were rare at Symes. Similar patterns were observed for alleles – of the 43 alleles observed, six were absent from Symes while only one was absent from Stone.

This study of *E. coli* in house mice also suggests that there are important interactions occurring between the dynamics of *E. coli* populations in their primary and secondary habitats. Further studies concerning the composition and transmission dynamics of *E. coli* in and between primary and secondary habitats would be valuable.

ACKNOWLEDGEMENTS

This study could not have been undertaken without the assistance of Grant Singleton and other members of the Rodent Control group of the Division of Wildlife and Ecology, Commonwealth Science and Industry Research Organization. The technical assistance of Arianne Lowe, Frances FitzGibbon and Leonie Moyle was invaluable. This study was funded by the Australian Research Council.

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Received 2 September 1996; revised 24 January 1997; accepted 7 February 1997.