The Evolutionary Consequences of Restrictions on Gene Flow: Examples from Hydrobiid Snails

D. J. Colgan
W. F. Ponder
The Australian Museum
P.O. Box A285
Sydney South, Australia 2000

ABSTRACT

The evolutionary consequences of restrictions on gene flow are discussed in relation to the population genetic structure and speciation of four Australian hydrobiid snail faunas. The studied faunas comprise: (1) the brackish water genus *Tatea;* (2) species of *Fluvidona* in freshwater streams at Wilsons Promontory; (3) species of *Fonscochlea* and *Trochidrobia in* artesian springs near Lake Eyre in South Australia; and (4) species of an undescribed genus at Dalhousie Springs in northern South Australia, another arid zone artesian spring complex. Gene flow in these hydrobiids is very variable. It is high in *Tatea,* relatively high at Dalhousie Springs and extremely low in Wilsons Promontory and the Lake Eyre springs. Levels in the latter two faunas are similar despite a great disparity in geographic area. In these four faunas, the detail of gene flow patterns is complex, emphasising the dependence of population structure on the interaction of current and historical factors. This is illustrated by speciation patterns, the numbers of species and their distributions usually correlating well with observed levels of gene flow. However, there are examples, among species groups with comparable, but very low gene flow, in which some taxa have undergone speciation yet others have not.

The data were analysed using F-statistics and the private allele frequency approaches. Whilst the qualitative conclusions from the two approaches were generally similar, the exceptions usually indicated (on biogeographic grounds), that the F-statistics approach is the more reliable estimator of gene flow. The private alleles approach is dependent on a coincidence of the scale of sampling with the biological scale of population subdivision. Intensive sampling schemes, as utilised in our studies, tend to find even rare alleles in more than one population even though they may be quite restricted in geographical distribution. An analytical method for treating conditional allelic frequencies would not be as sensitive to this problem as the private alleles approach.

Key words: Gene flow, snails, freshwater, isozymes, Hydrobiidae, evolution, population structure.

INTRODUCTION

The evolutionary fate of populations is largely determined by two sets of factors. The first may be characterised as coping factors —those determining the survival of a population. This set includes external pressures, such as predation, parasitism and disease, extremes of, or changes in, climate, competition from other species or resource diminution. It also includes endogenous properties such as the amount of local inbreeding or the capacity of the breeding system to engender genetic recombination. The second set may be characterised as isolating factors —those factors which affect the evolutionary history of a population relative to other, originally con-specific, populations. In general, this history depends on how effective gene flow is in overcoming differentiation inevitably arising from genetic drift or responses to local selection. Conversely, speciation processes are contingent on an "evolutionarily sufficient" restriction of gene flow between populations that have successfully accommodated the first set of factors. These two sets of factors are, however, also inter-related. For instance, it may be only through the introduction of a novel gene from another area that a population is enabled to withstand a climatic change.

Many studies have shown that speciation is directly related to reductions in gene flow (reviewed by Grant, 1980; Porter, 1990). It is difficult, however, if not impossible, to predict what restrictions on gene flow over what period of time constitute evolutionarily significant barriers. The importance of the evolutionary consequences of restriction on gene flow is such that its estimation remains a goal of many experimental studies *(e.g.* Skibinski *et al.,* 1983; Waples, 1987; Johnson *et al.,* 1988; Mitton *et al.,* 1989; Arter, 1990; Porter, 1990; Preziosi & Fairbairn, 1992). There is also continuing interest in the development of mathematical models for the analysis of gene flow *(e.g.* Slatkin, 1985a; Barton & Slatkin, 1986; Slatkin & Barton, 1989) and/or the the genetical subdivision of species (Cavalli-Sforza & Feldman, 1990).

We have been investigating the hydrobiid gastropod faunas of a variety of habitats to characterise their taxonomic and population genetic structure. We were particularly interested in how biological and environmental differences between these faunas are reflected in the

degree of genetic divergence and the amount of genetic exchange between populations, and in the evolutionary consequences of those differences. A priori, factors such as the biological ability to disperse, the geographic scale of the system, weather patterns, topography and the accessibility and suitability of the habitat for potential biological dispersal agents might all be expected to play a part in determining levels of gene flow.

Gene Flow

In this paper we define gene flow as the genetically-effective transmission of alleles between extant discrete populations and between various parts of the range within species in which population boundaries cannot be discerned or do not occur. We do not regard re-colonization after local extinction as an example of gene flow (agreeing with Endler, 1973; Grant, 1980;—but contrast Slatkin 1985b). Re-colonization, or original colonization simply increases the number of populations of a species, other processes being required for phylogenetic consequences. Generally, continued gene exchange will homogenize original and derived populations. Significant differentiation requires persistent marked reductions in gene flow.

Any estimate of gene flow made on the basis of allelic frequencies confounds factors operating during two distinct phases of the differentiation process. Firstly, the establishment of a population implies a sampling process which may cause differences in frequency arrays (Carson & Templeton, 1984; Wool, 1987). Secondly, the differences reflect subsequent patterns of gene flow and differential selection as well as any current trends. In the discussion below, we usually make the assumption that the comparisons between estimates of gene flow in different biological situations reflect differences in only one of these two confounded factors in any given case. This may not always be an accurate description of biological reality. High allelic frequency differences may result from a divergent initial founder effect or from a subsequent reduction in gene flow, differential selection or any combination of these three factors. Direct methods of estimating gene flow, requiring observation of the mating success and/or fertility of known immigrant individuals, largely overcome these problems. The labour and practical difficulies involved in making such estimates is such, however, that indirect methods are usually pursued (Slatkin, 1985b; Johnson *et al.*, 1988).

The Measurement of Gene Flow

Both methods of estimating gene flow which are used here measure the parameter *Nm,* where *N* is the (effective) size of each sub-population and m is the probability that a gamete in the offspring generation is an immigrant to the sub-population where it occurs. Three principal models of population structure have been developed as mathematical abstractions to provide a theoretical framework for measuring *Nm:*

(A) The Island model (Wright, 1931), in which each of an infinite number of discrete sub-populations receives migrants at random from other sub-populations. The geographic distance between populations does not affect the rate of gene flow between them.

(B) The Stepping Stone model (Kimura & Weiss, 1964), in which gene flow occurs between a population and its immediate neighbours in one or two dimensional geographic arrays. Gene flow does not occur directly between two populations which are not immediate neighbours.

(C) Models in which the population is considered to be continuously distributed in one or two dimensions, with the degree of genetic differentiation of individuals separated by a given distance determined by the levels of gene flow.

The accuracy of these models' approximation to population structure will vary. If the studied species has high vagility, the requirement of the Island model that each sub-population exchanges migrants with all others will be a more accurate approximation than if the vagility is low. Conversely, the Stepping Stone model's restriction on migration between populations which are not near neighbours is more likely to be accurate if the studied species has low vagility.

F-Statistics

The overall inbreeding coefficient can be partitioned into components reflecting non-random breeding (F_{IS}) and the effects of between sub-population differentiation (F_{ST}) (Wright, 1951). Under the infinite island model of population subdivision, if the migration rate is small, then (Wright, 1951):

$$F_{ST} \quad (1 + 4Nm)^{-1}$$

A variety, indeed almost a plethora, of alternative methods for the calculation of quantities very similar or identical to F_{ST} have been suggested (Wright, 1951, 1978; Nei, 1973; Nei & Chesser, 1983; Cockerham, 1969; Weir & Cockerham, 1984). Many of these were developed in response to complications of the original two-allele per locus situation studied by Wright. It can be shown that these are usually encompassed by natural extensions of Wright's approach. Others attempt to take account of relaxation of the simplifying assumptions (negligible selection, mutation, *etc.)* made in Wright's analyses. The various methods have been widely reviewed (e.g. Chakraborty & Leimar, 1987; Weir, 1990) and the differences between them shown, generally, to be of second-order significance. Moreover, both analytical (Slatkin, 1985b) and simulation (Slatkin & Barton, 1989) studies tend to emphasise the qualitative similarities between F_{ST} variables defined under either the Island or Stepping Stone Models. Where estimates of gene flow given below are based on F_{ST}, this will be indicated by $Nm(F_{ST})$.

Conditional Allelic Frequencies

There are two main methods of analysing gene flow using the approaches of Slatkin (1981, 1985a). The first requires that the occupancy rate for an allele be determined. This is the number of sub-populations in which the allele is found, divided by the total number of sub-populations. The conditional average frequency of the allele is the average of its frequencies in those sub-populations where it actually occurs. Levels of gene flow between sub-populations are visualised by graphing the conditional average frequency of each allele against occupancy rate. Such representations can be useful in comparison of the levels of gene flow in different taxa *(e.g.* Govindaraju, 1989) but, in the absence of an analytical theory, can be used to provide numerical estimates only by making analogies with the results of computer simulations (Johnson *et al.*, 1988). In the second method, attention is restricted to private alleles , *i.e.* those found in only one sub-population. Slatkin's simulations (1985a) found that, in both Island and Stepping Stone Models, the conditional average frequency of private alleles *(p(1))* is approximately linearly related to the migration rate by the expression:

$$\log_{10}(\bar{p}(1)) \quad a\log_{10}(Nm) + b$$

where a and *b take* values dependent on the number of individuals sampled from each sub-population. One claimed advantage of this approach is that its estimates of migration rates are theoretically only slightly dependent on mutation or the many types of selection which might operate (Barton & Slatkin, 1986; Slatkin & Barton, 1989). Estimates of gene flow based on the frequency of private alleles given below are designated as *Nm(p(1)).*

The Family Hydrobiidae

Small prosobranch snails of the world-wide family Hydrobiidae are the most diverse freshwater gastropods, with nearly 400 generic names currently in use (Kabat & Hershler, 1993). Commonly, in Australia, freshwater Hydrobiidae occupy small streams or springs. The populations in these isolated or semi-isolated habitats show varying degrees of differentiation because of an apparent inability to disperse readily. A low level of dispersal may be possible, for example by birds or even flying insects (Rees, 1965; Boeters, 1979, 1982).

Other genera inhabit brackish or estuarine waters. Genetic data have been used to test hypotheses based on morphological criteria in such taxa (Lassen, 1979; Davis *et al.*, 1988, 1989; Ponder & Clark, 1988; Ponder *et al.*, 1991). They tend to have large geographic ranges, partly because some have a planktonic marine larval phase, but also because they live in tidal marshland habitats where they are potentially readily transported by birds.

In the remainder of this paper, we will concentrate on our recent investigations of three freshwater hydrobiid radiations at Wilsons Promontory, in the Lake Eyre supergroup of the South Australian Mound Springs and in the Dalhousie Springs complex at the north of South Australia. For comparative purposes, we will often refer to our studies of the brackish water (usually estuarine) genus *Tatea* (Ponder *et al.,* 1991). The fauna of the Lake Eyre spring supergroup has been formally described (Ponder *et al.,* 1989) and that of Dalhousie Springs has been briefly reported on with respect to shell morphology (Ponder, 1989). The Wilsons Promontory study will be described in detail in a forthcoming publication (Ponder *et al.,* 1994).

MATERIALS AND METHODS

Summary information regarding collecting sites, *etc.* can be found in Appendix 2. More detail is provided for *Tatea* in Ponder *et al.* (1991), the Wilsons Promontory *Fluvidona* in Ponder *et al.* (1994), the Lake Eyre springs in Ponder *et al.* (1989) and on Dalhousie Springs in Zeidler and Ponder (1989). Genotypic data are available from the senior author. Data for *Tatea* and *Fluvidona* on allozymic frequencies, observed heterozygosities and the various environmental parameters which were measured are given in Ponder *et al.* (1991, 1994). Similar data for the other faunas will be presented separately for each system.

Standard methods for cellulose acetate electrophoresis were used (Hebert & Beaton, 1989, Ponder *et al.,* 1991). Individual snails were homogenized with 10-30 μl (mean 20 μl) of buffer, providing enough sample for up to 12 gels. Because of their small size, it was not possible to examine each snail for all enzymes. Where more than one locus is shown below as encoding the same enzyme, each was designated numerically in order of decreasing mobility. Allozymes identified for each locus are designated in the same way. The enzymes scored for each species, or species grouping, together with abbreviations used, Enzyme Commission Numbers, and number of presumptive loci are listed in Appendix 1. There were some differences between taxa in the number of loci that were electrophoretically interpretable. These are specified in Appendix 1. The computer packages BIOSYS-1 (Swofford & Selander, 1981), NTSYS (Rohlf, 1990) and PHYLIP, version 3-4 (Felsenstein, 1989) were used to assist analysis.

All taxonomic groupings treated here were initially analysed without assuming any hierarchical structure of the populations. F_{ST}, conditional allozymic frequencies and private allelic frequencies were calculated. Populations were then clustered in hierarchies, as described below and as detailed in Appendix 2. Components of overall genetic differentiation were obtained for this clustering using the WRIGHT78 step of BIOSYS. Allozymic frequencies in taxonomic units at each intermediate level of the clustering were calculated after pooling the data from the sub-units included in the same group. The pooled data were used to estimate F_{ST} values and conditional (and private) allelic frequencies for units at this intermediate level. This approach has a statistical tendency to reduce the variance in gene frequencies (and hence

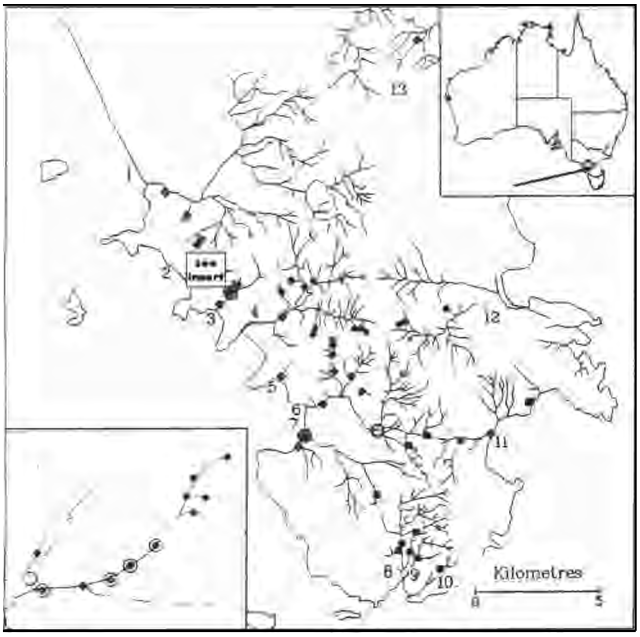

Figure 1. Map of Wilsons Promontory showing major drainages and the distribution of *Fluvidona* species. The area covered is shown by the dot at the head of the arrow on the inset map of Australia. The other inset shows a detail of Whisky Creek. Locations of straight-sided snails are shown by 0, the MPI 3 convex by MPI 4 by ◆ and MPI 5 by ◇. Drainages: (1) Darby River; (2) Whisky Creek; (3) Squeaky Creek; (4) Tidal River; (5) Titania Creek; (6) Growler Creek; *(7)* Frasers Creek; (8) Roaring Meg; (9) Picnic Creek; (10) First Bridge Creek; (11) Freshwater Creek; (12) Blackfish Creek; and (13) Chinamans Creek.

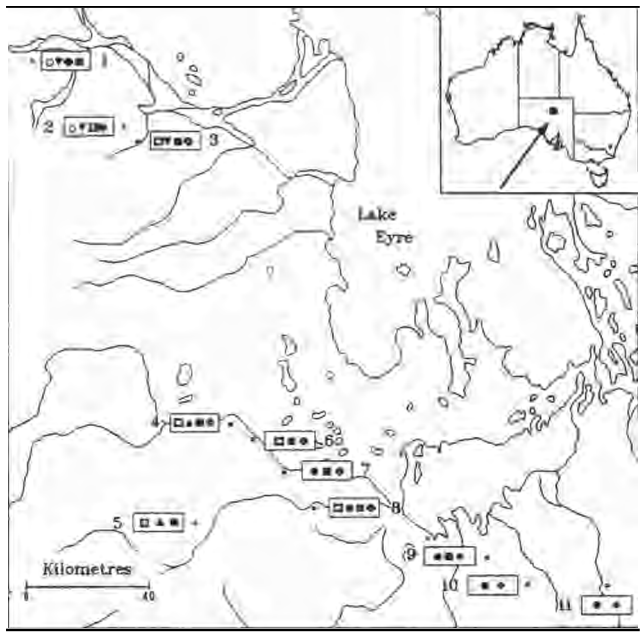

Figure 2. Map of the Lake Eyre mound springs showing the distribution of *Fonscochlea* and *Trochidrobia* species. The area covered is indicated on the inset of Australia. The site of a spring group is indicated by an "x". Presence of a species in the group is indicated by for *F. accepta,* ◇ for *F. aquatica,* ■ for *F. zeidleri,* ▲ for *F. billakalina,* ▼ for *F. variabilis,* • for *T. punicea,* 0 for *T. minuta* and 0 for *T. smithi.* The spring groups are: (1) Freeling; (2) Outside; (3) Twelve Mile; (4) Strangways; (5) Billakalina; (6) Beresford/Warburton; (7) Coward/Jersey/Elisabeth/Kewson; (8) Blanche Cup; (9) Hermit Hill; (10) Davenport; and (11) Welcome.

increase the estimate of *Nm)* to a degree dependent on levels of variation between samples pooled into the same unit. If the variation is merely a sampling artefact then the procedure will increase accuracy of *Nm* estimation. But if the variation is due to biological subdivision of the populations, then the estimate should be regarded more as an upper limit on the degree of gene flow. A priori, it is not possible to decide which of these two alternatives is correct as we do not know what constitutes an effectively panmictic unit in these hydrobiids. A second effect of the pooling procedure is that alleles which are found in more than one population and hence not "private" in the original subdivision, may be regarded as private at higher clustering levels if they are there restricted to only one unit. Again, this reflects the uncertainty about the biological structure of the population. Pooling data may not always resolve this uncertainty but patterns in such analyses will usually be informative about population structure to at least some extent.

The sample sizes used in the studies varied from locus to locus and from population to population. The average sample per locus is shown in Appendix 2. When *Nm* was estimated from the conditional frequencies of private alleles, the parameter values for a sample size of 25 were taken from Barton and Slatkin (1986). Alternative parameter values did not, however, significantly affect numerical estimates of gene flow.

THE STUDY AREAS

Wilsons Promontory

Wilsons Promontory (Figure 1), the southern-most part of the Australian mainland (39°S, 146°28'W), consists of granite hills up to 754 m with many permanent streams and rivers fed by high rainfall (>1000 mm per year). Its geological and climatological setting are summarised by Wallis (1988) and Schmidt and Thornton (1992). The hydrobiid fauna (genus *Fluvidona)* of the Promontory comprises two endemic morphologically-recognisable species, the shells of one with straighter whorl outlines and one with more convex whorls. The latter morphospecies is divisible into three genetic species by very nearly fixed sympatric differences in the MPI phenotype, referred to as the MPI 3, MPI 4 and MPI 5 genetic species.

For hierarchical analyses of population structure, sites were grouped within streams, streams within catchments and catchments within species, providing two intermediate levels (streams, catchments) in an analysis. Populations in which hybrids were seen were ignored in the

analyses examining gene flow within genetic species (see Appendix 2).

South Australian Mound Springs

The springs, fed from the Great Artesian Basin of Australia, are of considerable limnological and conservation significance to the very arid area in which they occur (Ponder, 1986; Harris, 1993). The springs generally lie on the fringes of the Basin, where the aquifers abut impervious rock or lie near the surface. The Lake Eyre Supergroup, the most extensive group of springs associated with the Great Artesian Basin, extends about 400 km between Marree and Oodnadatta and provides virtually the only permanent water in the area. The area with springs is only rarely more than 20 km wide, so that the supergroup conforms quite well to a one dimensional, discontinuous model. The geological history of these springs is not well known. Estimates of the ages of some large (extinct) mounds range from late Miocene to Recent. These are probably at least Pleistocene (Wopfner & Twidale, 1976; Williams & Holmes, 1978; Thompson & Barnett, 1985) but the springs have probably been in the area much longer. The taxa presently inhabiting the springs may be relicts of more widespread forms from a generally wetter period in the Neogene or may represent faunas associated with these artesian springs through much of the Tertiary.

The predominant drainage pattern in the Lake Eyre basin is at right angles to the line of springs. Hence the transport of snails between spring groups by floods would be unlikely—although such transport could occur within spring groups. Spring nomenclature and grouping used in this paper follows Ponder *et al.* (1989). The hydrobiid fauna consists of two endemic genera, *Fonscochlea* (five species) and *Trochidrobia* (four species) (Ponder *et al.*, 1989)

For hierarchical analyses, springs were compared within spring groups. The groups were then collected into clusters (see Figure 2, Appendix 2), the Southern cluster comprising springs between Welcome Springs and Hermit Hill, the Middle cluster comprising springs between the Blanche Cup complex and Strangways and the Northern comprising Outside, Twelve Mile and Freeling Springs. If species were found in more than one of these clusters, a second intermediate level was included in the hierarchy. This could not, however, be done for all species, *Fonscochlea accepta,* for instance being found only in the Southern cluster. The Southern cluster was divided into three groups: (1) Welcome Springs; (2) Davenport Springs; and (3) the Hermit Hill springs. The Middle cluster was divided into five groups: (1) Blanche Cup springs, (2) the group consisting of Coward, Kewson, Elizabeth and Jersey springs, (3) Billakalina; (4) Beresford and Warburton Springs; and (5) Strangways Springs. The Northern cluster was divided into two groups: (1) Twelve Mile and Outside springs; and (2) Freeling Springs.

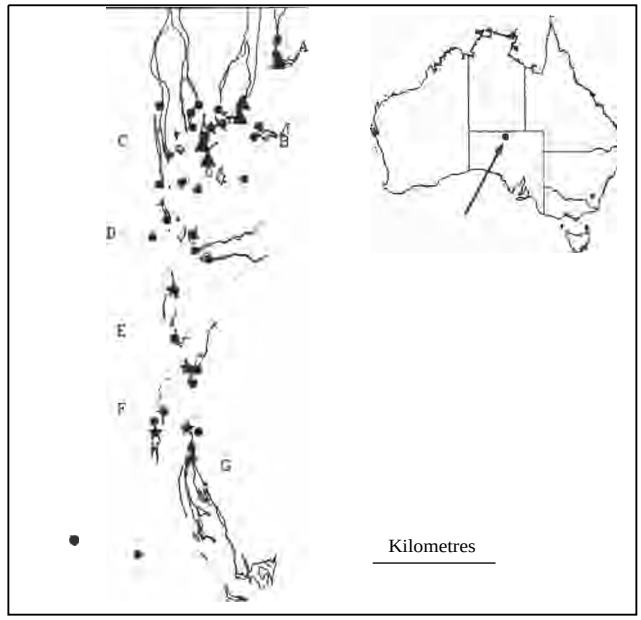

Figure 3. Map of the Dalhousie Springs showing the distribution of the globular (A), pupiform (●) and *Fluvidona-like* snails (*). Spring groups are identified by letters near dashed boundaries.

Dalhousie Springs

This complex is another large group of arid zone springs in northern South Australia associated with the Great Artesian Basin (Figure 3). Aspects of its geology and biology are surveyed in a number of papers in Zeidler and Ponder (1989). The many springs in the complex occupy an area of about 70 km^2. They range in size from small nascent or senescent seeps, to actively flowing and, at the upper end of the size scale, to outlets (of about 140 L/sec) which feed pools of 50 m or more in width with outflow channels supporting wetland vegetation for up to 15 km (Smith, 1989). Their combined discharge accounts for 90-95% of the total produced by all South Australian artesian springs (Smith, 1989), and 41% of the overall output from Great Artesian Basin springs (Habermehl, 1982). They are well separated from springs of the Lake Eyre supergroup, the northernmost population of species from that region *(F. zeidleri)* being 140 km away. These springs are likely to be early Pleistocene in age (Krieg, 1989). Minor local overflow due to rare heavy rain may facilitate interspring transport. Major flooding is unlikely (Kotwicki, 1989).

Spring nomenclature and groupings used in this paper follow Zeidler and Ponder (1989), except as specified below. Eight main groups of springs, designated A to H are recognised (Figure 3, Appendix 2). C is divided into four sub-groups, and D into two. Herein, group H will be treated as comprising two groups, because H3 is well separated from H1. We have also split E into two subgroups containing, respectively, (1) E5 and El and (2)

Table 1. Estimates of Nm derived from the average frequency of private alleles or from the average F_{ST} at various clustering levels. The overall estimates assume no population hierarchy. The next two columns are estimates from data pooling within the first intermediate hierarchical level and the final two columns are for pooling within the second hierarchical level (where applicable). The upper figure in each cell is the observed value of the variable. The lower figure is the value of Nm calculated from the observation.

	Overall		Level 1 pooling		Level 2 pooling	
Species	F_{ST}	$\bar{p}(1)$	F_{ST}	$\bar{p}(1)$	F_{ST}	$\bar{p}(1)$
Fluvidona (straight-sided)	0.130	0.444	0.120	0.375	0.108	0.348
	0.776	0.313	0.491	0.417	0.589	0.468
Fluvidona (convex) MPI 3	0.086	0.535	0.110	0.523		
	0.872	0.217	0.570	0.228		
MPI 4	0.075	0.681	0.199	0.628	0.081	0.462
	1.104	0.117	0.205	0.148	0.967	0.291
MPI 5	0.161	0.240	0.091	0.282	0.073	0.254
	0.295	0.791	0.791	0.637	1.157	0.734
Fonscochlea accepta	0.116	0.207	0.028	0.110		
	0.525	0.958	6.036	2.023		
F. aquatica	0.110	0.781	0.128	0.756	0.149	0.412
	0.570	0.070	0.439	0.081	0.338	0.357
F. zeidleri	0.360	0.728	0.289	0.750	0.155	0.625
	0.074	0.093	0.108	0.083	0.316	0.150
F. variabilis	0.196	0.792	0.300	0.783	0.173	0.688
	0.211	0.066	0.101	0.069	0.261	0.113
F. billakalina	0.044	0.263	0.226	0.300		
	2.769	0.700	0.165	0.583		
Trochidrobia punicea	0.143	0.600	0.098	0.580	0.160	0.470
	0.363	0.167	0.696	0.175	0.299	0.282
T. smithi	0.426	0.730	0.265	0.584	0.224	0.346
	0.055	0.092	0.125	0.178	0.167	0.472
T. minuta	0.086	0.375	0.244	0.429		
	0.872	0.417	0.144	0.381		
Dalhousie (globular)	0.159	0.321	0.038	0.105	0.028	0.082
	0.302	0.529	3.565	2.131	6.036	2.799
Dalhousie (pupiform)	0.180	0.413	0.015	0.296	0.014	0.228
	0.244	0.355	17.706	0.595	19.942	0.846
Dalhousie *(Fluvidona-like)*	0.296	0.670	0.300	0.492		
	0.104	0.123	0.101	0.258		

E2, E7 and E8. Generally, for hierarchical analyses, springs were clustered into sub-groups, sub-groups into groups and groups into the species. Three hydrobiid species were recognised in our genetic studies. The globular and pupiform species belong to an undescribed endemic genus, the third species (also endemic) being tentatively included in the widespread genus *Fluvidona.* Separate analyses were performed for each species.

RESULTS

The levels of gene flow in the four *Fluvidona* taxa from Wilsons Promontory are extremely low as shown by the overall F-statistics in Table 1. The smallest F_{ST} value is for the convex MPI 5 genetic species. At 0.240, this is, however, near the higher end of the range previously found for gastropods over comparable geographic scales (Gould & Woodruff, 1986, 1990; Johnson *et al.,* 1988). The overall F_{ST} values for the straight-sided *Fluvidona* species and the convex MPI 3 and MPI 4 genetic species are very high. The levels of migration suggested by these values range down to 0.117 for the MPI 4 genetic species, implying that the fraction of a deme which is replaced by immigrants each generation (m = 0.117/N) is very small. The estimates of $Nm(F_{ST})$ values based on the pooling of data from individual samples may be complicated by the likelihood that the pooled data do not represent single populations. The trends in the estimates are, however, very similar to those based on single populations. Those for the MPI 4 genetic species are higher than those for other *Fluvidona* taxa, but still suggest that

Table 2. Variance components in the hierarchical F-statistic analyses. The F_{XY} figures indicate that variance ascribable to variation in the specified X variable *(e.g.,* population) within the specified Y variable *(e.g.,* spring group). Where two intermediate levels are used for a species in the hierarchy, all six cells are filled. Where one level is used only three cells are filled. The top figure in each cell is the calculated F_{XY} value and the bottom, the percentage of the total variance comprised by this.

	Variance components					
X variable: Population Y variable: Species	Population Level 1	Population Level 2	Population Total	Level 1 Level 2	Level 1 Total	Level 2 Total
Fluvidona (straight-sided)	0.226 13	0.434 27	0.426 26	0.269 17	0.257 16	0.015 1
Fluvidona (convex) MPI 3	0.005 0	0.374 17	0.570 26	0.371 17	0.567 26	0.312 14
MPI 4	0.152 6	0.539 20	0.679 25	0.456 16	0.622 23	0.305 11
MPI 5	0.052 6	0.190 20	0.255 27	0.145 15	0.214 23	0.081 9
Fonscochlea accepta	0.122 35		0.173 49		0.058 16	
F. aquatica	0.217 7	0.744 23	0.771 24	0.673 21	0.707 22	0.105 3
F. zeidleri	0.346 12	0.677 23	0.718 24	0.506 17	0.568 19	0.127 4
F. variabilis	0.382 12	0.599 19	0.781 24	0.352 11	0.646 20	0.454 14
F. billakalina	0.101 22		0.220 48		0.133 29	
Trochidrobia punicea	0.118 6	0.262 13	0.574 28	0.164 8	0.518 25	0.423 21
T. smithi	0.745 48		0.719 46		0.100 6	
T. minuta	0.059 8		0.358 49		0.318 43	
Dalhousie (globular)	0.288 33	0.303 35	0.284 33	0.021 2	-0.006 -1	-0.027 -3
Dalhousie (pupiform)	0.304 24	0.363 28	0.384 30	0.085 7	0.115 9	0.032 2
Dalhousie *(Fluvidona-like)*	0.538 43		0.656 53		0.049 4	

Nm is less than one. Values for MPI 4 are all higher than for the individual sample estimation, marginally so for the pooling of samples within tributaries and notably for the pooling into catchment based units. Even so, the data suggest that a catchment receives less than one migrant from another catchment in every three generations.

Restrictions on gene flow are also suggested by analyses of the conditional frequency of private allozymes. Estimates of migration rates based on these data are much greater than those based on F_{ST} and differ in the relative rates ascribed to the different taxa. The latter situation is particularly notable in MPI 4 which apparently has the highest rate of inter-population migration among all four species, whereas its $Nm(F_{ST})$ is the lowest.

The components of variance due to differentiation between taxonomic units at different hierarchical levels are presented in Table 2. Although comparison of these values is complicated by varying proportions of the populations being pooled at each level, some trends can be observed. Particularly striking is the concordance between the three MPI genetic species, where in each case almost half of the variation is explained by differences between tributaries or between catchments. This contrasts with the straight-sided *Fluvidona* where only one third of the variability is explained by such differences.

We have investigated gene flow in eight of the nine Lake Eyre mound springs hydrobiids, *Trochidrobia inflata* being found in only two of our sample sites. As can be seen in Figure 2 and Appendix 2, the distributions of these species vary markedly in size. *Fonscochlea accepta* is restricted to the Southern cluster of springs, *F. billakalina* to the central cluster and *T. minuta* to the northern. The range of the other species extends into more than one spring cluster, with *F. zeidleri* and *F. variabilis* being found in all three clusters. The apparent levels of gene flow between the populations of the species reflect this variability in range. *F. accepta* has high gene flow, with $Nm(F_{ST})$ between spring groups being more than two. Conversely, gene flow in *F. aquatica,* the snail which is an ecological replacement for *F. accepta* in the central

Table 3. Estimates of F_{ST} or G_{ST} in gastropods. Measures using G_{ST} (Nei, 1973) are indicated by an asterisk. Geographic scale is the distance between the extremes of the sampled range. References are: (1) Johnson and Black (1984a,b); (2) Brown (1991); (3) Mitton *et al.* (1989); (4) Campton *et al.* (1992); (5) Grant and Utter (1988); (6) Day (1990); (7) Chambers (1980); (8) Jarne and Delay (1990); (9) Mulvey *et al.* (1988); (10) Bandoni *et al.* (1990); (11) Johnson *et al.* (1988); (12) Gould and Woodruff (1986); and (13) McCracken and Brussard (1980).

Species and reference	No. of loci	No. of samples	Scale (km)	F_{ST} mean
Marine species				
(1) Siphonaria jeanae	4	1	10	0.002
	4	28	2,500	0.004
(2) *Haliotis* rubra	12	18	5,000	0.022
(3) Strombus gigas	7	21	5,000	0.076
(4) Strombus gigas	4	4	500	0.011*
	4	14	5,000	0.023*
(5) Nucella lamellosa	2	12	0.1	0.021
	2	30	1,000	0.286
(6) Nucella lapillus	8	6	0.5	0.015
	8	10	10	0.092
	8	15	20	0.195
Freshwater species				
(7) Goniobasis (2 species)	14	12	1,000	0.408
(8) Lymnaea peregra	6	4	50	0.018
(9) Biomphalaria glabrata	13	6	1,000	0.805
(10) Biomphalaria pfeifferi	7	12	500	0.589
Terrestrial species				
(11) Partula taeniata	17	22	20	0.279
P. suturalis	16	23	20	0.168
(12) Cerion (New Providence)	8	36	30	0.143
(13) Triodopsis albolabris	2	7	500	0.255

and northern clusters, is quite low, *Nm* being 25 times less between spring groups. In these large aquatic *Fonscochlea,* approximately the same relative levels of gene flow are indicated by the estimates derived from conditional allelic frequencies. Using F_{ST} for estimation, a similar pattern is shown in comparisons of the smaller aquatic species *F. billakalina* and *F. variabilis,* with gene flow between spring groups in the former being nine times the level in the latter. In *Trochidrobia,* gene flow inferred from F_{ST} statistics between spring groups in *T. minuta* is twice as high as it is in the other two species of the genus. In these latter two sets of comparisons, however, the estimates derived from conditional allelic frequencies do not show the same pattern as the F_{ST} estimates. *F. billakalina* has a similar $Nm(F_{ST})$ to *F. variabilis* and the value for T. *punicea* is almost five times as great as that for *T. minuta.*

The components of variation due to different hierarchical levels are strikingly similar in the aquatic *F. aquatica* and the amphibious *F. zeidleri.* There is some disagreement as to the level of $Nm(F_{ST})$ between spring clusters for these species, but otherwise estimates of interpopulation migration in these species are remarkably concordant. The concordance is significantly less for $Nm(\tilde{p}(1))$. The components of variation are also similar in two other species *(F.* variabilis and *T.* punicea) from the Lake Eyre mound springs. Interestingly, the pattern of variation of this pair differs from that of the two large aquatic *Fonscochlea,* showing much greater between-spring divergence.

Estimated $Nm(F_{ST})$ between populations at Dalhousie Springs is in the higher reaches of the ranges observed in these studies, in both the pupiform and globular species. This trend is even more marked for inter-group migration as assayed at higher hierarchical levels. Inter-spring subgroup migration is higher in the globular snails than in any other level-one pooling, and the estimate for the pupiform snails is exceeded only by *F.* accepta and *F.* billakalina. The calculated migration rates betwen spring groups are higher for both the globular and pupiform Dalhousie radiations than for any other taxon in our studies. As expected, the proportion of variation explained by differences at the lower hierarchical levels is very high in comparison to our other studies. Indeed, virtually all of the variation in the globular snails is due to differentiation of populations within spring-subgroups, within spring groups, or within the overall spring complex (and not of spring-subgroups within groups, etc.). The levels of gene flow estimated from *Nm(p(1))* for the two higher hierarchical levels are extremely high in the context of the present results. To the extent that these estimates are credible, they reinforce the suggestion that gene flow is high in the Dalhousie Springs complex, at least as compared to the other study sites.

DISCUSSION

Our hydrobiid studies emphasise the dependence of population structure on a wide range of interacting biological and environmental factors which must be considered in historical terms. The three hydrobiid faunas we have treated extensively in this paper, and the previously studied *Tatea* (Ponder *et al.*, 1991) differ (either certainly or probably) in such biological characteristics as size, thermal and salinity tolerances and desiccation resistance. These various hydrobiids also occupy different types of *habitat. Tatea* occupies essentially continuous habitat. Wilsons Promontory *Fluvidona* and the Dalhousie Springs snails have habitats which are discontinuous, with relatively small distances between suitable areas. The Lake Eyre fauna is in discontinuous, widely separated habitat.

Before discussing our results in detail, some preliminary comparisons may be made to reinforce that gene flows actually do differ between the faunas. The Wilsons Promontory radiation occupies a much smaller geographic area than the Lake Eyre fauna, with the exception of *Fonscochlea accepta.* The markedly higher levels of gene flow in *F. accepta* might suggest that the underlying processes are more efficient in this taxon. Conversely, such relativity emphasises the very restricted levels of gene flow in the Wilsons Promontory snails. This is also suggested by comparisons of these *Fluvidona* species with the Lake Eyre *F. aquatica, F. variabilis* and *F. zeidleri.* The MPI 4 and MPI 3 genetic species do have a slightly higher $Nm(F_{ST})$ than the *Fonscochlea* species but this is a minor difference when the disparity between their ranges is considered. These *Fonscochlea* species each have linear extents well over an order of magnitude larger than the MPI 3 and MPI 4 *Fluvidona* (over 200km as opposed to less than 20km).

Population Structure in Quasi-Continuous Aquatic Habitats

Although the vagaries of ocean currents may reduce gene flow to or from a particular area (Todd *et al.,* 1988; Mitton *et al.,* 1989), marine snails with planktonic (and especially planktotrophic) larvae have wide natural distributions in which variation between local populations is only a minor fraction of overall genetic diversity (Table 3). This pattern is found *in Nassarius obsoletus* (Gooch *et al.,* 1972), *Littorina littorea* (Berger, 1973; Janson, 1987), *Siphonaria jeanae* (Johnson & Black, 1984a, 1984b), the species with planktotrophic larvae among the *Crepidula* studied by Hoagland (1984), *Strombus gigas* (Mitton *et al.,* 1989; Campton *et al.,* 1992) and *Haliotis rubra* (Brown, 1991) which has a lecithotrophic larva. Species distributions in these marine gastropods tend to be either widely separated, often in conjunction with geographic barriers to gene flow, or to be broadly sympatric *(e.g. Littorina* - Berger, 1973; Janson, 1987; *Crepidula* - Hoagland, 1984) presumably reflecting past allopatric speciation and subsequent dispersal into sympatry. The latter pattern was observed in our studies of *Tatea* (Ponder *et al.,* 1991). This genus, predominantly estuarine with an assumed free-swimming larval stage, has a very wide distribution, being found throughout temperate Australia. Its two species *T. rufilabris* and *T. huonensis* are sympatric over virtually all of this range. There are exceptions to these patterns of speciation in some groups with specialised feeding patterns which have high species densities (Vermeij, 1987).

Groups with direct larval development or brooding should have reduced gene flow, greater differentiation and, conceivably, higher likelihood of speciation. The first two predictions have been borne out in studies of *Littorina saxatilis* (Snyder & Gooch, 1973; Janson, 1987) and *Nucella lamellosa* (Grant & Utter, 1988). That a short planktonic phase increases rates of speciation is less certain. *Lit torina saxatilis* does have a number of closely-related sibling species (Janson, 1987; Johannesson, 1988; Sundberg *et al.,* 1990) and *N. lamellosa* may represent a species complex (Grant & Utter, 1988). However, *N. lamellosa,* as presently recognised, has one of the largest geographic ranges of North Pacific gastropods.

Population Structure in Discontinuous Aquatic Habitats

There have been major investigations of gene flow and the genetic structure of two groups of the freshwater gastropods in the caenogastropod genus *Goniobasis.* Chambers (1978, 1980) investigated species from Florida and Dillon and Davis (Dillon & Davis, 1980; Dillon, 1984) those from the border regions of Virginia-North Carolina. Three main results are relevant. (1) There is a high degree of genetic divergence between populations within the same drainage system indicating low levels of gene flow. (2) There are larger differences between drainage systems, reflecting an even smaller likelihood of inter-drainage gene flow. (3) Identified taxa tend to remain allopatric or parapatric, with geographically-restricted ranges, suggesting that dispersal after speciation is limited. Dillon (1988) provides direct information on rates of gene flow in transplanted *G. proxima* populations. These are about 15-20 m upstream and 5-10 m downstream (per year), the discrepancy in movement rates being caused by the behavioural tendency of freshwater (Dillon, 1988) (and even riparian—Arter, 1990) snails to crawl upstream in compensation for down current drift.

The findings for *Goniobasis* are not true of all freshwater snails, as shown by studies of genetic variation in basommatophoran pulmonates. Dispersal in these snails is often assisted by self-fertilisation (Mimpfoundi & Greer, 1989; Bandoni *et al.,* 1990) and species distributions are generally wide-ranging. Measurement of gene flow is often hampered because of extremely low levels of genetic variation (Mimpfoundi & Greer, 1989; Jame & Delay, 1991). Where flow can be assessed, as in *Biomphalaria straminea* (Woodruff *et al.,* 1985), *B. camerunensis* (Mimpfoundi & Greer, 1990), *B. pfeifferi* (Bandoni *et al.,* 1990) or *Bulinus cernicus* (Rollinson *et al.,*

Berger, E. 1973. Gene-enzyme variation in three sympatric species of *Littorina.* Biological Bulletin 145:83-90.

Boeters, H.D. 1979. Species concepts of prosobranch freshwater molluscs in western Europe, 1. Malacologia 18:57-60.

Boeters, H.D. 1982. Species concepts of prosobranch freshwater molluscs in western Europe, 2. Malacologia 22:499-504.

Brown, L.D. 1991. Genetic variation and population structure in the Blacklip Abalone, *Haliotis* rubra. Australian Journal of Marine and Freshwater Reseasrch 42:77-90.

Cain, A. J. and J.D. Currey. 1963. Area effects in *Cepaea.* Philosophical Transactions of the Royal Society of London B 246: 1-181.

Cameron, R.A. and P.J. Dillon. 1984. Habitat stability, population histories and patterns of variation in *Cepaea.* Malacologia 25:271-290.

Campton, D.E., C.J. Berg, Jr., L.M. Robinson and R.A. Glazer. 1992. Genetic patchiness among populations of queen conch *Strombus gigas* in the Florida Keys and Bimini. Fishery Bulletin, U.S. 90:250-259.

Carson, H.L. and A.R. Templeton. 1984. Genetic revolutions in relation to speciation phenomena:the founding of new populations. Annual Review of Ecology and Systematics 15:97-131.

Cavalli-Sforza, L.L. and M. Feldman. 1990. Spatial subdivision of populations and estimates of genetic variation. Theoretical Population Biology 37:3-25.

Chakraborty, R. and 0. Leimar. 1987. Genetic variation within a sub-divided population. *In:* Ryman, N. and F. Utter (eds.). Population genetics and fishery management. University of Washington Press, Seattle, p. 90 - 120.

Chambers, S.M. 1978. An electrophoretically detected sibling species of *"Goniobasis floridensis* (Mesogastropoda: Pleuroceridae). Malacologia 17:157-162.

Chambers, S.M. 1980. Genetic divergence between populations of *Goniobasis* (Pleuroceridae) occupying different drainage systems. Malacologia 20:63-81.

Clarke, B. and J. Murray. 1969. Ecological genetics and speciation in land snails of the genus *Partula.* Biological Journal of the Linnean Society 1:31-42.

Cockerham, C.C. 1969. Variance of gene frequencies. Evolution 23:72-84.

Crow, J.F. and K. Aoki. 1984. Group selection for a polygenic trait: estimating the degree of population subdivision. Proceedings of the National Academy of Sciences U.S.A. 81: 6073-6077.

Davis, G. M., V. Forbes and G. Lopez. 1988. Species status of northeastern American *Hydrobia* (Gastropoda: Prosobranchia): Ecology, morphology and molecular genetics. Proceedings of the Academy of Natural Sciences of Philadelphia 140:191-246.

Davis, G. M., M. McKee and G. Lopez. 1989. The identity of *Hydrobia truncata* (Gastropoda: Hydrobiinae): Comparative anatomy, molecular genetics, ecology. Proceedings of the Academy of Natural Sciences of Philadelphia 141: 333–359.

Day, A.J. 1990. Microgeographic variation in allozyme frequencies in relation to the degree of exposure to wave action in the dogwhelk *Nucella lapillus* (L.) (Prosobranchia: Muricacea) Biological Journal of the Linnean Society 40:245-261.

R.T., Jr. 1984. Geographic distance, environmental difference, and divergence between isolated populations. Systematic Zoology 33:69-82.

Dillon, R.T., Jr. 1988. The influence of minor human disturbance on biochemical variation in a population of freshwater snails. Biological Conservation 43:137-144.

Dillon, R.T., Jr. and G.M. Davis. 1980. The *Goniobasis* of Southern Virginia and Northwestern North Carolina: Genetic and shell morphometric relationships. Malacologia 20:83-98.

Endler, J.A. 1973. Gene flow and population differentiation. Science 179:243-250.

Felsenstein, J. 1989. PHYLIP - Phylogeny Inference Package (Version 3.2). Cladistics 5:164-166.

Gooch, J.L., B.S. Smith and D. Knupp. 1972. Regional survey of gene frequencies in the mud snail *Nassarius obsoletus.* Biological Bulletin 142:36-48.

Gould, S.J. and D.S. Woodruff. 1986. Evolution and systematics of Cerion (Mollusca: Pulmonata) on New Providence Island: a radical revision. Bulletin of the American Museum of Natural History 182:389-490.

Gould, S. J. and D.S. Woodruff. 1990. History as a cause of area effects: an illustration from *Cerion* on Great Inagua, Bahamas. Biological Journal of the Linnean Society 40: 67-98.

Govindaraju, D.R. 1989. Estimates of gene flow in forest trees. Biological Journal of the Linnean Society 37:345-357.

Grant, V. 1980. Gene flow and the homogeneity of species populations. Biological Zhentralblatt 99:157-169.

Grant, W.S. and F.M. Utter. 1988. Genetic heterogeneity on different geographic scales in *Nucella lamellosa* (Prosobranchia, Thaididae). Malacologia 28:275-287.

Habermehl, M.A. 1982. Springs in the Great Artesian Basin, Australia - their nature and significance. Bureau of Mineral Resources, Canberra, Geology and Geophysics, Report No. 235.

Harris, C. 1993. Mound springs: South Australian conservation inititives. Rangelands Journal 14:157-173

Hebert, P.D.N. and M.J. Beaton. 1989. Methodologies for Allozyme analysis using Cellulose Acetate Electrophoresis. Helena Laboratories, Austin. 32 pp.

Hoagland, K.E. 1984. Use of molecular genetics to distinguish species of the gastropod genus *Crepidula* (Prosobranchia: Calyptreidae). Malacologia 25:607-628.

Janson, K. 1987. Allozyme and shell variation in two marine snails *(Lit torina,* Prosobranchia) with different dispersal abilities. Biological Journal of the Linnean Society 30:245-256.

Jarne, P. and B. Delay. 1990. Population genetics of *Lymnaea peregra* (Muller) (Gastropoda: Pulmonata) in Lake Geneva. Journal of Molluscan Studies. 56:317-321.

Jarne, P. and B. Delay. 1991. Population genetics of freshwater snails. Trends in Ecology and Evolution 6:383-386.

Johanessen, K. 1988. The paradox of Rockall: Why is a brooding gastropod *(Littorina saxatilis)* more widespread than one having a planktonic larval dispersal stage *(L. littorea)?* Marine Biology 99:507-513.

Johnson, M.S. and R. Black. 1984a. The Wahlund effect and the geographic scale of variation in the intertidal limpet *Siphonaria* sp. Marine Biology 70:295-302.

Johnson, M.S. and R. Black. 1984b. Pattern beneath the chaos: The effect of recruitment on genetic patchiness in an intertidal limpet. Evolution 38:1371-1383.

Johnson, M.S., B. Clarke and J. Murray. 1977. Genetic variation and reproductive isolation in *Partula.* Evolution 31: 116-126.

Johnson, M.S., J. Murray and B. Clarke. 1986a. An electrophoretic analysis of phylogeny and evolutionary rates in

the genus *Partula* from the Society Islands. Proceedings of the Royal Society of London B 227:161-177.

Johnson, M.S., J. Murray and B. Clarke. 1986b. High genetic similarities and low hetreozygosities in land snails of the genus *Samoana* from the Society Islands. Malacologia 27: 97-106.

Johnson, M.S., B. Clarke and J. Murray. 1988. Discrepancies in the estimation of gene flow in *Partula*. Genetics 120: 233-238.

Johnson, M.S., O.C. Stine and J. Murray. 1984. Reproductive compatibility despite large-scale genetic divergence in *Cepaea nemoralis*. Heredity 53:655-665.

Kabat, A.R. and R. Hershler. 1993. The prosobranch snail family Hydrobiidae (Gastropoda: Rissooidea): Review of classification and supraspecific taxa. Smithsonian Contributions to Zoology. Smithsonian Contributions to Zoology, 547:1-94.

Kimura, M. and G.H. Wiess. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49:561-576.

Kotwicki, V. 1989. Floods in the western Lake Eyre basin and their impact on Dalhousie Springs. *In:* Zeidler, W. and W.F. Ponder (eds.). Natural history of Dalhousie Springs. South Australian Museum, Adelaide, p. 41-45.

Krieg, G.W. 1989. Geology. *In:* Zeidler, W. and W.F. Ponder (eds.). Natural history of Dalhousie Springs. South Australian Museum, Adelaide, p. 19-26.

Lamotte, M. 1951. Recherches sur la structure génétique des populations naturelles de *Cepaea nemoralis* L. Bulletin Biologique de France, Supplement 35:1-239.

Lassen, H.H. 1979. Electrophoretic enzyme patterns and breeding experiments in Danish mud snails (Hydrobiidae). Ophelia 318:38–87.

McCracken, G.F. and P.F. Brussard. 1980. The population biology of the White-lipped land snail, *Triodopsis albolabris:* genetic variability. Evolution 34:92-104.

Mimpfoundi, R. and G.J. Greer. 1989. Allozyme comparisons among species of the *Bulinus forskalii* group (Gastropoda: Planorbidae) in Cameroon. Journal of Molluscan Studies 55:405-410.

Mimpfoundi, R. and G.J. Greer. 1990. Allozyme variation among populations of *Biomphalaria camerunensis* (Boettger, 1941) (Gastropoda: Planorbidae) in Cameroon. Journal of Molluscan Studies 56:373-381.

Mitton, J.B., C.J. Berg, Jr., and K.S. Orr. 1989. Population structure, larval dispersal and gene flow in the Queen Conch, *Strombus gigas* of the Caribbean. Biological Bulletin 177:356-362.

Mulvey, M., M.C. Newman and D.S. Woodruff. 1988. Genetic differentiation among West Indian populations of the schistosome-transmitting snail *Biomphalaria glabrata*. Malacologia 29:309-317.

Murray, J. and B. Clarke. 1980. The genus *Partula* on Moorea: speciation in progress. Proceedings of the Royal Society of London B 211:83-117.

Nei, NI. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences U.S.A. 70:3321-3323.

Nei, M. and R.K. Chesser. 1983. Estimation of fixation indices and gene diversification. Annals of Human Genetics 47: 253-259.

Ochman, H., J.S. Jones and R.K. Selander. 1983. Molecular area effects *in Cepaea*. Proceedings of the National Academy of Sciences U.S.A. 80:4189-4193.

Ponder, W.F. 1986. Mound springs of the Great Artesian Basin. *In:* DeDeckker, P. and W.D. Williams (eds.). Limnology in Australia CSIRO, Melbourne and W. Junk, The Hague, p. 403-420.

Ponder, W.F. 1989. Mollusca. *In:* Zeidler, W. and W.F. Ponder (eds.). Natural history of Dalhousie Springs. South Australian Museum, Adelaide, p. 71-80.

Ponder, W. F. and G.A. Clark. 1988. A morphological and electrophoretic examination of *'Hydrobia buccinoides',* a variable brackish-water gastropod from temperate Australia (Mollusca: Hydrobiidae). Australian Journal of Zoology 36:661-689.

Ponder, W.F., D.J. Colgan and G.A. Clark. 1991. The morphology, taxonomy and genetic structure of *Tatea* (Mollusca: Gastropoda: Hydrobiidae), estuarine snails from temperate Australia. Australian Journal of Zoology 39:447-497.

Ponder, W. F., R. Hershler and B. Jenkins. 1989. An endemic radiation of hydrobiid snails from artesian springs in northern South Australia: their taxonomy, physiology, distribution and anatomy. Malacologia 31:1-140.

Ponder W.F., D.J. Colgan, G.A. Clark, A.C. Miller and T. Terzis. 1994. Microgeographic genetic and morphological differentiation of freshwate snails - a study on the Hydrobiidae of Wilsons Promontory, Victoria, southeastern Australia. Australian Journal of Zoology, In press.

Porter, A.H. 1990. Testing nominal species boundaries using gene flow statistics: the taxonomy of two hybridising Admiral butterflies *(Limenitis:* Nymphalidae). Systematic Zoology 39:131-147.

Preziosi, R.F. and D.J. Fairbairn. 1992. Genetic population structure and levels of gene flow in the stream-dwelling waterstrider, *Aquarius (= Gerris) remigis* (Hemiptera: Gerridae). Evolution 46:430-444.

Rees, W.J. 1965. The aerial dispersal of Mollusca. Proceedings of the Malacological Society of London 36:269-282.

Rohlf, F. J. 1990. NTSYS-pc. Numerical taxonomy and multivariate analysis system. Version 1.60. Exeter Software, New York.

Rollinson, D., R.A. Kane, A. Warlow and V.R. Southgate. 1990. Observations on genetic diversity of *Bulinus cernicus* (Gastropoda: Planorbidae) from Mauritius. Journal of Zoology 222:19-26.

Schmidt, E.R. and I.W.B. Thornton. 1992. The Psocoptera (Insecta) of Wilsons Promontory National Park, Victoria. Memoirs of the Museum of Victoria, 53,137-220.

Skibinski, D.O.F., J.A. Beardmore and T.F. Cross. 1983. Aspects of the population genetics of *Mytilus* (Mytilidae: Mollusca) in the British Isles. Biological Journal of the Linnean Society 19:137-183.

Slatkin, NI. 1981. Estimating levels of gene flow in natural populations. Genetics 99:323-335.

Slatkin, NI. 1985a. Rare alleles as indicators of gene flow. Evolution 39:53-65.

Slatkin, NI. 1985b. Gene flow in natural populations. Annual Review of Ecology and Systematics 16:393-430.

Slatkin, NI. and N.H. Barton. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43:1349-1368.

Smith, P.C. 1989. Hydrogeology. *In:* Zeidler, W. and W.F. Ponder (eds.). Natural history of Dalhousie Springs. South Australian Museum, Adelaide, p. 27-39.

Snyder, T.P. and J.L. Gooch. 1973. Genetic differentiation in *Littorina saxatilis* (Gastropoda). Marine Biology 22:177-182.

Sundberg, P., A.J. Knight, R.D. Ward and K. Johannesson.

1990. Estimating the phylogeny in mollusc *Littorina saxatilis* (Olivi) from enzyme data: methodological considerations. Hydrobiologia 193:29-40.

Swofford, D.L. and R.B. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity. 72: 281-283.

Thomson, R. and S. Barnett. 1985. Geology, geomorphology and hydrogeology. *In:* Greenslade, J., L. Joseph and A. Reeves (eds.). South Australia's mound springs. Nature Conservation Society of South Australia, Adelaide, p. 3–26.

Todd, C.D., J.N. Havenhand and J.P. Thorpe. 1988. Genetic differentiation, pelagic larval transport and gene flow between local populations of the intertidal marine mollusc *Adalaria* proxima (Alder and Hancock). Functional Ecology 2:441-451.

Vermeij, G. 1987. The dispersal barrier in the tropical Pacific: implications for molluscan speciation and extinction. Evolution 41:1046-1058.

Wallis, G.L. 1988. Wilsons Promontory. *In:* Clark, I. and B. Cook (eds.). Victorian Geology Excursion Guide. Australian Academy of Science, Canberra, 353-372.

Waples, R.S. 1987. A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41:385-400.

Weir, B. 1990. Intraspecific differentiation. *In:* Hillis, D.M. and C. Moritz (eds.). Molecular systematics. Sinauer Associates, Sunderland, p. 373-410.

Weir, B. and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

White, M.J.D. 1978. Modes of speciation. W. H. Freeman and Co., San Francisco. pp. 455.

Whitlock, M.C. 1992. Temporal fluctuations in demographic parameters and the genetic variance among populations. Evolution 46:608-615.

Williams, A.F. and J.W. Holmes. 1978. A novel method of estimating the discharge of water from mound springs of the Great Artesian Basin, Central Australia. Journal of Limnology 38:262-272.

Woodruff, D.S., M. Mulvey and M.W. Yipp. 1985. Population genetics of *Biomphalaria straminea* in Hong Kong. Journal of Heredity 76:355-360.

Wool, D. 1987. Differentiation of island populations: a laboratory model. American Naturalist 129:188-202.

Wopfner, H. and C.R. Twidale. 1967. Geomorphological history of the Lake Eyre Basin. In: Jennings, J.N. and J.A. Mabbutt (eds.). Landform studies in Australia. Australian National University Press, Canberra, p. 118-143.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97-159.

Wright, S. 1951. The genetical structure of populations. Annals of Eugenics 15:323-354.

Wright, S. 1978. Evolution and the genetics of populations. Vol. 2. The theory of gene frequencies. University of Chicago Press, Chicago.

Zeidler, W. and W.F. Ponder. 1989. Natural history of Dalhousie Springs. South Australian Museum, Adelaide. pp. 138.

Appendix I. The numbers of interpretable loci in the taxonomic groups of studied snails. The columns give the enzyme name, its abbreviation, E.C. number and the number of loci which were interpretable in the designated taxa. Column A is Wilsons Promontory *Fluvidona;* B is *Fonscochlea accepta;* C *F. aquatica;* D *F. zeidleri;* E *F.* variabdis; F *Trochidrobia punicea;* G *T. smithi* and *T. minuta;* H, the Dalhousie Springs globular and pupiform snails; and I, the Dalhousie *Fluvidona*-like species.

Enzyme	Abbreviation	E.C. no.	Loci								
			A								
Alkaline phosphatase	ALKP	3.1.3.1								1	1
Aspartate aminotransferase	AAT	2.6.1.1	2	2	2	2	2	2	1	2	2
Enolase	ENO	4.2.1.11	1	1	1	1	1	1	1	1	1
Esterase	EST	3.1.1.1	1	3	2	3	3	3	1	3	3
Fumarate hydratase	FH	4.2.1.2		1	1	1	1	2		1	
β-Galactosidase	GAL	3.2.1.23								1	
Glycerol-3-phosphate dehydrog.	GPD	1.1.1.8					1	1	1	1	1
Glucosephosphate isomerase	GPI	5.3.1.9	1	1	1	1	1	1	1	1	1
Glutamate-pyruvate transaminase	GPT	2.6.1.2	2	1	2	2	2		2		2
Hexokinase	HK	2.7.1.1		1	1	1					
Hydroxybutyrate dehydrogenase	HI3DH	1.1.1.30						1	1	1	1
Isocitrate dehydrogenase	IDH	1.1.1.42	2	2	2	1	2	2	2	2	2
Leucine aminopeptidase	LAP	3.4.11	1							1	
Malate dehydrogenase	MDH	1.1.1.37	2	2	1	2	2	2	2	2	2
Mannosephosphate isomerase	MPI	5.3.1.8	1	1	1	1	2	1	1	2	1
Octopine dehydrogenase	ODH	1.5.1.15		1	1	1	1				
Peptidases: Phe-Pro substrate	PPR	3.4.11	1							1	1
: Len-Ala substrate	LAL	3.4.11	2					3	2	1	
: Val-Leu substrate	VL	3.4.11		1	1	1	2				
: Leu-Leu-Gly substrate	LGG	3.4.11		1	1	1	1			2	
Phosphoglucomutase	PGM	5.4.2.2	2	2	2	1	1	1	1	2	2
6-Phosphogluconate dehydrog.	6-PGDH	1.1.1.44	1	1	1	1	1	1	1	1	1
Purine nucleoside phosphorylase	PNP	2.4.2.1		1		1	1				
Sorbitol dehydrogenase	SDH	1.1.1.14	2	1	2	1	2	2			2
Triosephosphate isomerase	TPI	5.3.1.1	1	1	1	1	1	1	1	1	1
UDP glucose pyrophosphorylase	UDPG	2.7.7.9								1	1

Appendix 2. Hierarchical sample structure analysed for various hydrobiid species. The highest hierarchical levels are written flush to the left margin of each column. Lower levels are successively indented. Spring groups and watersheds are identified in figures 1 to 3. The average number of specimens scored for each locus is given after the sample designations.

Wilsons Promontory *Fluvidona* Hierarchy: Catchment-Stream-Site. 'MAIN' indicates the principal stream of the catchment, 'ONE', 'TWO', *etc.,* the tributaries. Sites within catchments are numbered, approximately, clockwise.

Straight-sided	
WHISKY CREEK	
LOWER	
WC1	12.64
WC3	8.36
UPPER	
WC5	12.09
WC6	8.27
WC7	11.41
SQUEAKY CREEK	
MAIN	
SC2	7.09
FRASER CREEK	
MAIN	
FC1	7.09
GROWLER CREEK	
MAIN	
GC10	7.55
Convex MP13	
GROWLER CREEK	
MAIN	
GC12	7.27
FIVE	
GC13	7.00
FRASER CREEK	
MAIN	
FC3	7.54
ROARING MEG	
MAIN	
RM1	7.54
RM3	8.54
ONE	
RM2	5.27
TWO	
RM4	13.64
FIRST BRIDGE CREEK	
MAIN	
FB1	7.54
PICNIC CHEEK	
MAIN	
PC1	7.32

Appendix 2. Continued.

FRESHWATER CREEK	
TWO	
FW3	6.41
WATERLOO BAY	
MAIN	
WB1	7.09
WB2	7.09
Convex MP14	
WHISKY CREEK	
MAIN	
WC6	16.09
WC7	13.55
WC8	12.95
WC9	12.99
WC10	13.95
WC11	13.90
WC12	27.95
TIDAL RIVER	
ONE	
TR4	38.45
TR5	8.63
THREE	
TR7	8.36
TR8	7.63
FOUR	
TR9	8.00
FIVE	
TR11	8.09
TR10	8.81
TWO	
TR2	8.63
SIX	
TR6	8.36
GROWLER CREEK	
MAIN	
GC2I	11.41
ONE	
GCI6	8.96
TWO	
GC6	7.93
THREE	
GC4	7.82
GC5	7.91
BLACKFISH CREEK	
ONE	
BC4	5.91
TWO	
BC6	5.91
THREE	
BC8	7.45

Appendix 2. Continued.

SQUEAKY CREEK	
MAIN	
SC2	7.36
ONE	
SC3	7.36
Convex MPI5	
CHINAMANS CREEK	
MAIN	
CC1	15.72
DARBY RIVER	
MAIN	
DR2	14.50
ONE	
DR3	13.96
TWO	
DR4	14.64
WHISKY CREEK	
MAIN	
WC1	12.55
WC2	10.00
WC4	11.64
WC5	11.64
TIDAL RIVER	
MAIN	
TR1	10.72
GROWLER CREEK	
MAIN	
GC25	3.34
FRESHWATER CREEK	
MAIN	
FW1	7.09
FRASER CREEK	
MAIN	
MN1	7.09
ONE	
FC1	7.09
SQUEAKY CREEK	
MAIN	
SC1	14.28

Lake Eyre Hydrobiidae Hierarchy: Region-Spring group-Site. Samples are identified by spring group initials and a number indicating south-north order in the entire Lake Eyre collections.

F. *accepta*	
WELCOME SPRINGS	
WS1	15.38
WS2	10.38
WS3	15.38

Appendix 2. Continued.

DAVENPORT SPRINGS	
DS4	13.92
DS5	10.38
DS6	13.62
HERMIT HILL	
HH7	15.42
HH8	10.38
HH9	15.38
HHIO	10.46
HHI 1	10.46
HH12	10.42
F. *aquatica*	
MIDDLE	
BLANCHE CUP	
BC13	9.70
BC15	9.85
BC16	9.85
COWARD/KEWSON	
CSI9	9.81
ES20	9.15
ES21	9.77
KH27	7.54
JS28	9.73
JE29	9.31
BERESFORD SPRINGS	
BS22	9.69
STRANGWAYS SPRINGS	
SS24	8.85
SS30	6.77
NORTH	
OUTSIDE SPRINGS	
OS25	8.85
TM26	9.00
FREELING SPRINGS	
FR31	10.73
FR32	9.73
F. *billakalina*	
BILLK	
BK18	7.92
STRANGWAYS	
SS24	8.00
SS30	9.13
F. *variabilis*	
SOUTH	
WELCOME SPRINGS	
WS2	9.80
DAVENPORT SPRINGS	
DS6	8.60
MIDDLE	
BLANCHE CUP	
BC13	9.70
BC14	10.44
BC15	10.20
BC16	7.92

Appendix **2.** Continued.

COWARD/KEWSON	
CS17	7.92
CS19	8.00
ES20	8.56
ES21	10.08
KH27	8.44
JS28	10.20
JE29	7.80
BERESFORD SPRINGS	
BS22	8.32
WA23	8.20
NORTH	
OUTSIDE SPRINGS	
OS25	8.52
TM26	8.00
FREELING SPRINGS	
FR31	7.75
FR32	7.96
F. zeidleri	
SOUTH	
HERMIT SPRINGS	
HH8	8.50
MIDDLE	
BLANCHE CUP	
BC13	7.54
BC14	10.25
COWARD/KEWSON	
CS17	10.25
CS19	8.75
ES20	10.21
ES21	9.96
KH27	7.88
JS28	10.17
JE29	8.33
BILLAKALINA	
BK18	8.75
BERESFORD SPRINGS	
BS22	10.04
WA23	10.25
STRANGWAYS SPRINGS	
SS24	10.09
NORTH	
OUTSIDE SPRINGS	
TM26	8.88
FREELING SPRINGS	
FR32	8.79
T. punicea	
SOUTH	
WELCOME SPRINGS	
WS1	8.00
WS3	9.46

Appendix **2.** Continued.

DAVENPORT SPRINGS	
DS4	9.00
DS5	8.92
DS6	8.00
HERMIT HILL	
HH7	8.00
HH8	8.00
HH10	7.96
HH12	8.00
MIDDLE	
BLANCHE CUP	
BC13	8.00
BC15	8.08
COWARD/KEWSON	
CS19	7.38
ES20	7.88
ES21	7.92
KH27	7.88
JS28	6.92
JE29	7.92
T. smithi	
MIDDLE	
BLANCHE CUP	
BC14	14.00
BILLAKALINA	
BK18	16.89
BERESFORD SPRINGS	
BS22	10.22
STRANGWAYS SPRINGS	
SS24	13.27
SS30	9.22
NORTH	
OUTSIDE SPRINGS	
OS25	15.33
TM26	11.89
T. minuta	
OUTSIDE SPRINGS	
OS25	8.44
FREELING SPRINGS	
FR31	13.61
FR32	11.56

Dalhousie Springs Hydrobiidae Hierarchy: Spring group-Sub-group-Site. Samples marked with a p are from the pool of large springs and those with o from the outflow.

Globular			
A	ONE	A1	6.64
		A3	8.57
		A8	19.57
	ONE	B1	11.43
	A	Ca1p	49.50
		Ca1o	37.07
		Ca1o	28.39
		Ca1o	33.54
		Ca9	32.25

Appendix 2. Continued.

	B	Cb2	6.93
		Cb2a	5.93
		Cb2b	6.29
	C	Ccl	6.07
		Cc3	6.50
	D	Cdlp	7.40
		Cdlp	7.00
		Cdlo	4.32
		Cdlo	7.00
		Cd2	14.25
		Cd8	5.21
Pupiform			
A	ONE	Al	18.89
		A2	21.29
		A3	6.11
		A6	17.82
		A8	34.11
B	ONE	B1	8.86
		B2	9.79
C	A	Ca2	29.79
		Ca3	26.29
		Ca5	33.25
		Ca7a	28.35
		Ca7b	30.71
		Ca8	17.79
		Ca12	30.18
		Cal3	28.25
	B	Cb4	8.21
		Cb5o	6.00
		Cb5p	17.86
	C	Ccl	6.43
		Cc4	16.43
		Cc8	8.32
	D	Cdlp	6.96
		Cd3	5.61
		Cd5	5.43
		Cd9	6.36

Appendix 2. Continued.

D	A	Dal	6.00
		Da2	17.64
		Da3	11.75
	B	Db1	6.00
		Db2	9.79
		Db4	5.96
E	A	E5	23.21
	B	El	19.85
		E2	9.21
		E7a	18.07
		E8	21.93
F	ONE	Fl	20.04
		F2	17.89
G	ONE	Ga2	26.07
		Ga3	33.14
		Ga4	15.00
		Ga6a	6.39
		Ga6b	14.07
H	ONE	H1	6.96
	TWO	H3	17.75
Fluvidona-like			
C		Cd11	4.16
		Elo	8.32
E		E3	4.16
F		F9	4.16
G		Gal	4.16
		Ga2o	4.16
		Ga6	4.16